Physiological Zoölogy

Vol. XIV OCTOBER 1941 *No. 4*

STUDIES IN POPULATION PHYSIOLOGY. X. INTERSPECIFIC COMPETITION IN POPULATIONS OF GRANARY BEETLES[1]

(Fourteen figures)

THOMAS PARK, ELLA VIRGINIA GREGG, AND CATHARINE Z. LUTHERMAN
Hull Zoölogical Laboratory, University of Chicago

INTRODUCTION

POPULATIONS can be studied experimentally by using either a single-species or a mixed-species culture. In the former case attention centers on the analysis of a comparatively simple type of biological association. There is an attempt to assay as many mass relations between the organisms and their environment as possible. These studies are "intraspecific" and are typified by the investigations of Johnson (1933) and Reich (1936) on microörganisms; of Pearl (1932) and Bodenheimer (1938) on *Drosophila* populations; of Chapman (1928) and Park (1941) on *Tribolium;* and of Allee and Evans (1937) on *Arbacia punctulata.* The mixed-species or "interspecific" studies are concerned primarily with analysis of those competitive (or co-operative) relations established within a population when several species with essentially similar ecological requirements live together. On an experimental basis this analysis demands (1) that an empirical description of the growth and trend of the entire population shall be worked out and (2) that the factors associated with or causing the observed trends shall be understood. The two most-considered interactions between species have been predator-prey relationships and the competition of two species for food and *Lebensraum.* This interest was focused by the theoretical writings of Lotka (1934), Volterra (1928), and Nicholson (1933); by the obvious implications of interspecific behavior for evolution and speciation; and by the observations of ecologists on competitive phenomena in natural populations. However, with a few exceptions, there has been little attempt to analyze competition or the "struggle for existence" experimentally. The notable exceptions are the work of Gause (1934) on microörganisms; of Gause, Smaragdova, and Witt (1936) and Smaragdova (1936) on the mites *Cheyletus* (predator) and *Aleuroglyphus* (prey); of Omori (1939) on two species of *Cimex;* and of Vladimerova and Smirnov (1938) on the competition between two genera of flies (*Musca* and *Phormia*) for food.

[1] The present investigation was aided by a grant from the Dr. Wallace C. and Clara A. Abbott memorial fund of the University of Chicago. The authors are pleased to acknowledge their indebtedness for this grant. We acknowledge also a grant from the Ridgeway Fund of the University of Chicago to defray in part the cost of publication.

In this paper another experimental analysis of interspecific competition will be presented. This is a long-time project. Although the data discussed here are based on more than two years' continuous research, they are essentially descriptive in character. They are suggestive in that new lines of attack are opened for future experimentation. This is not an apology. In population work it is particularly desirable to build a picture of growth trends based on the entire population before proceeding to detailed analyses of causal factors.

MATERIALS AND METHODS

In an adequate interspecific population study the following are important technical desiderata: first, it must be feasible to culture all species in the same medium in a restricted space; second, the medium should be reproducible both as to quality and as to quantity; third, the husbandry of each species must be relatively simple; fourth, a method of taking a census must exist (this method cannot be so drastic that it injures the organisms, and it must yield accurate results); and, fifth, it must be possible to reconstitute the population after each census and to maintain the medium and the control cultures as long as the experiment warrants. For the present experiments three species of granary beetles have been selected. They appear to fulfil a number of the requirements set forth above. The beetles are *Tribolium confusum*, the flour beetle; *Gnathoceros cornutus*, the broad-horned flour beetle, and *Trogoderma versicolor*. The first two are Tenebrionids; the latter a Dermestid. All three species came from closely inbred stocks, the *Tribolium* from the authors' laboratory and the *Trogoderma* and *Gnathoceros* from the Stored Products Research Laboratory of Slough, England.

Medium.—Some difficulty was encountered in devising a medium that could be sifted for census-taking and, at the same time, that would be suitable nutritionally for all three species of beetles. *Tribolium* lives well in a finely milled flour (for husbandry see Park, 1934). This is ideal since the flour is readily sifted and, with a minimum of effort, eggs, larvae, pupae, and imagoes can be collected for counting. *Gnathoceros* and *Trogoderma* require coarse as well as fine particles in their medium. This complicates the census since it is tedious to segregate the stages when the larger particles are present. However, as there was no way to avoid this difficulty a medium of the following composition was made up:

70 parts (by weight) of sifted whole-wheat flour
28 rolled oats
2 sifted, dry yeast

The flour ("Lenfesty" whole wheat) and the yeast (Fleischmann's dry brewer's yeast, *Saccharomyces cerevisiae*) are sifted through a silk bolting cloth with 82 meshes to the inch. This sieve retains all metamorphic stages of all three species. The coarser middlings and bran are discarded, and the fine flour (with the yeast) is used. The rolled oats ("Quaker" brand) are sifted through a coarse bolting cloth (17 meshes to the inch) to remove broken flakes and dust. Then flour, yeast, and oats are added together and gently but diligently stirred until the medium becomes a homogeneous mixture consisting of a finely milled component (flour and yeast) smaller than any of the beetles' stages and a coarse component (oat flakes) larger than any of the beetles' stages.

Census procedure.—In making counts it is feasible to sift the medium through a number of bolting-cloth sieves of different-sized mesh in order to segregate the beetles and

their metamorphic stages according to size. This procedure facilitates counting and can be outlined as follows in chronological sequence:

1. The total medium is passed through a coarse bolting-cloth sieve (17 meshes to the inch). This removes from the culture the larger flakes as well as imagoes, pupae, and late instar larvae. These flakes are set aside for later examination.
2. The residue is sifted through a fine sieve (82 meshes to the inch). This takes out the major part of the medium as a powder from which all remaining beetle stages (eggs, early and middle instar larvae) have been removed and retained by the sieve. The powder is discarded.
3. The remaining medium is sifted through a sieve (30 meshes to the inch) and the larvae (middle instar) thus collected and set aside.
4. The residue is sifted through a sieve (38 meshes to the inch) and the larvae (early and middle instar) thus collected and set aside.
5. The residue is sifted through a sieve (48 meshes to the inch) and the larvae (early instar) and some eggs thus collected and set aside. The residue contains essentially no eggs or larvae of *Tribolium* and *Gnathoceros*, and the few that are present are removed. *Trogoderma* eggs are elongated cylinders, and some of them pass through the 48-mesh sieve. Unfortunately, these eggs are lost.
6. The sifting is now concluded. The five portions containing medium and beetles are then worked over by hand, and all the beetles' stages except the eggs are segregated for counting. The eggs cannot be counted because they are of the same size as the finest residue and thus cannot be separated. The residue containing eggs is not discarded but returned to the population. Thus the eggs, although not counted, are not lost except in the case of *Trogoderma*, where some eggs pass through the 48-mesh sieve. No residue is discarded until it is examined under the binocular dissecting microscope for larvae or other stages that might have been overlooked.
7. After counting, the following items, depending on the mating in question, are recorded:
   a) For every mating the age of the culture, date of examination, and initials of observers.
   b) For all three species the number of larvae and pupae.
   c) For *Tribolium* the total number of imagoes (these imagoes cannot be sexed).
   d) For *Gnathoceros* and *Trogoderma* the number and sex of the imagoes.
   e) For *Trogoderma* the number and sex of the *dead* imagoes (the significance of this record will appear later).
   f) For all populations any special remarks that might be pertinent.
8. After counting and recording, the new population is started by returning all stages to *fresh* medium and gently redistributing them through their environment.

It is apparent from this description that the census method used in these experiments is a *total* rather than a *sampling* method. This is something of a novelty in population work. It rules out obviously the type of error associated with any sampling technique. At the same time the populations are large enough to avoid the criticism of small numbers.

Environmental control.—Since the experiments are a study in biotic competition, it is necessary to control external environmental factors so far as possible. When this is done,

it is legitimate to say that, to a large extent, the results observed are an expression of intraculture dynamics rather than extra-culture differentials. To this end the temperature, light, humidity, quantity and quality of the medium, and handling are controlled rather carefully. All populations are maintained in a dark, thermostat-regulated incubator at a temperature of 29° (±0.5° variation on the average). The relative humidity in the incubators varies from 60 to 70 per cent on the mean, although during July and August it is consistently above this figure. In the winter the humidity is raised by placing trays containing sand in the bottom of the incubator. All populations are kept on the top shelf of the same incubator so that what variation does exist is common for all. The culture containers are wide-mouth bottles, 100 mm. high and 70 mm. in diameter. One hundred grams of medium is placed in each culture bottle. The medium is allowed to come into temperature and moisture equilibrium with the incubator's environment since it is prepared and bottled a week or more before using. Handling is standardized by sifting mechanically and by following the same routine in examination of all cultures. The beetles are handled as gently as possible with brush and spatula.

The experimental design.—The experiments reported in this paper are designed so that (1) there are records of population growth of *Tribolium*, *Gnathoceros*, and *Trogoderma* grown as pure cultures (single species); these constitute controls; (2) there are records of population growth when three species are cultured in all combinations of twos with both species initially seeded in equal imaginal density; and (3) there are records of population growth when all three species are cultured in all combinations of twos with one species initially seeded in a higher imaginal density than its competing mate. The experiments are set up as follows:

Mating	Code Letter
44 *Tribolium* males × 44 *Tribolium* females	A
44 *Gnathoceros* males × 44 *Gnathoceros* females	B
44 *Trogoderma* males × 44 *Trogoderma* females	C
22 *Tribolium* males & 22 *Tribolium* females × 22 *Gnathoceros* males & 22 *Gnathoceros* females	D
22 *Tribolium* males & 22 *Tribolium* females × 22 *Trogoderma* males & 22 *Trogoderma* females	E
22 *Gnathoceros* males & 22 *Gnathoceros* females × 22 *Trogoderma* males & 22 *Trogoderma* females	F
38 *Tribolium* males & 38 *Tribolium* females × 6 *Gnathoceros* males & 6 *Gnathoceros* females	G
38 *Tribolium* males & 38 *Tribolium* females × 6 *Trogoderma* males & 6 *Trogoderma* females	H
38 *Gnathoceros* males & 38 *Gnathoceros* females × 6 *Tribolium* males & 6 *Tribolium* females	I
38 *Gnathoceros* males & 38 *Gnathoceros* females × 6 *Trogoderma* males & 6 *Trogoderma* females	J
38 *Trogoderma* males & 38 *Trogoderma* females × 6 *Tribolium* males & 6 *Tribolium* females	K
38 *Trogoderma* males & 38 *Trogoderma* females × 6 *Gnathoceros* males & 6 *Gnathoceros* females	L
15 *Tribolium* males & 15 *Tribolium* females × 15 *Gnathoceros* males & 15 *Gnathoceros* females × 15 *Trogoderma* males & 15 *Trogoderma* females	M

In the present paper a particular mating will be referred to by its code letter. When it is necessary to indicate a replication, a superscript will be used. Thus F^2 designates the second culture started with *Gnathoceros* and *Trogoderma* in equal density.

The experimental procedure can be summarized as follows: (1) All cultures are maintained under the same environmental conditions; (2) all cultures are started with 88

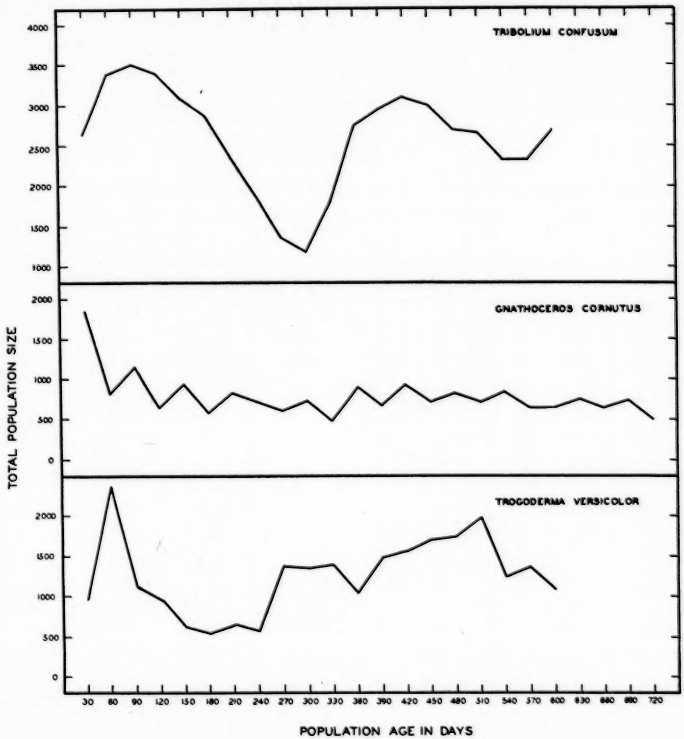


FIG. 1.—Population growth of control cultures of *Tribolium confusum*, *Gnathoceros cornutus*, and *Trogoderma versicolor*. Curves based on means.

young imagoes in 100 gm. of medium (except the M-mating which is a special case); (3) all cultures are examined every 30 days. At that time records of numbers are taken, and the beetles are returned to their appropriate bottle containing fresh medium. Conditioning of the flour (see Park, 1941, and Park and Woollcott, 1937) thus is kept purposely from being an important variable in these matings.

In the following discussion we shall consider, first, the growth of the control or single-species populations; second, the competition in the mixed-species cultures and its end effects; and, third, the biological implications of these data.

TABLE 1*

COMPOSITION AND TOTAL SIZE OF *Tribolium* CONTROLS RELATIVE TO HIGH AND LOW DENSITIES

Age Interval (Days)	Composition of Total Population in Percentages									Total Population (Larvae, Pupae, and Imagoes)			
	Larvae			Pupae			Imagoes						
	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	*n*
30–180 (1st high-density period)	61.1±1.50	13.4±1.06	21.9	5.3±1.02	9.1±0.72	171.7	33.6±2.11	18.8±1.49	55.9	3146.30± 74.8	665.8±52.0	21.2	36
210–330 (1st low-density period)	67.1±1.19	9.7±0.84	14.4	4.2±0.29	2.4±0.20	57.1	28.7±1.26	10.3±0.89	35.9	1686.56± 72.0	640.6±50.0	37.9	30
360–510 (2d high-density period)	70.3±0.57	5.1±0.40	7.2	7.1±0.50	4.5±0.35	63.4	22.6±0.51	4.6±0.36	20.4	2861.40± 61.3	545.2±43.3	19.0	36
540–600 (2d low-density period)	63.5±1.27	8.0±0.89	12.6	12.1±0.76	4.8±0.53	39.7	24.4±0.84	5.3±0.59	21.7	2459.61±108.0	680.9±76.5	27.7	18
0–600 (total period)	65.5±0.73	11.8±0.52	18.0	7.2±0.36	5.8±0.25	80.5	27.3±0.83	13.6±0.59	49.8	2538.46± 61.4	997.5±43.4	39.3	120

* The sampling parameter used throughout this paper is the probable error.

GROWTH OF SINGLE-SPECIES POPULATIONS

Tribolium confusum.—The data on *Tribolium* population growth are based on census observations made every 30 days over a 600-day span on six different cultures. The growth trend computed as a mean of these records is shown in Figure 1. This figure immediately suggests the interesting point that these *Tribolium* populations, after reaching their asymptote, do not maintain themselves at that place but, instead, begin a slow decline and, after reaching a low point at about 300 days, start to build up again. A second peak is reached around 420 days, which is followed by another but lesser decline. In order to appreciate better the magnitude of this effect the 600 days' observations are divided into four periods as follows: the first high-density period (30–180 days); the

TABLE 2

ANALYSIS OF *Tribolium* CONTROLS WITH LOWEST DENSITIES SET EQUAL

Mean Age	A^1	A^2	A^3	A^4	A^5	A^6	Mean	Deviation from Mean Low
85.....	4,234	3,208	3,374	4,425	3,649	2,395	3,547	+2,667
115.....	3,790	2,897	3,010	3,918	3,195	2,723	3,256	+2,376
145.....	3,439	2,998	2,790	3,825	2,822	3,230	3,184	+2,304
175.....	3,039	2,840	2,925	3,687	2,556	2,512	2,927	+2,047
205.....	2,472	1,824	2,596	3,012	2,163	2,448	2,419	+1,539
235.....	1,894	1,837	1,812	2,556	1,704	1,990	1,965	+1,085
265.....	1,056	1,073	1,149	1,931	1,029	1,465	1,284	+ 404
Low 295.....	740	734	1,021	850	623	1,314	880	0
325.....	1,651	2,024	1,243	1,498	1,764	2,216	1,733	+ 853
355.....	2,862	3,445	2,520	2,246	3,355	2,734	2,860	+1,980
385.....	3,370	3,647	2,890	2,992	3,809	2,561	3,211	+2,331
415.....	3,232	2,914	3,456	2,319	2,604	2,138	2,777	+1,897
445.....	2,774	3,249	3,494	3,604	2,218	2,663	3,000	+2,120
475.....	3,094	2,385	3,042	3,467	2,273	2,399	2,777	+1,897
505.....	2,655	1,930	2,544	2,647	1,478	2,849	2,350	+1,470

first low-density period (210–330 days); the second high-density period (360–510 days); and the second low-density period (540–600 days). For each of these periods the percentage composition[2] of larvae, pupae, and imagoes is calculated, as well as the mean population size. These data appear in Table 1. The following points emerge from study of this table:

1. The total population size of the first period (3,146 beetles per 100 gm. medium) is about double that of the 210–330-day period (1,686 beetles).
2. While there are suggestions of a second high (2,861 beetles) and a second low (2,459 beetles) at 360–510 days and 540–600 days, respectively, it is obvious that the oscillations are dampened. The relative variation as measured by the coefficient of variability is somewhat higher for the low- than for the high-density periods.

[2] Throughout this paper the term "composition" will be used in this specific sense, namely, to denote for a single species the percentage composition of the total culture in terms of larvae, pupae, and imagoes.

3. The mean population size attained by *Tribolium* under these conditions is 2,538 beetles per 100 gm. of medium. The significance of this figure will become apparent when it is compared with that for *Gnathoceros* and *Trogoderma* controls.
4. Larvae form the bulk of the population, with imagoes next and pupae last. The larval counts are also the least, and the pupal the most, variable. Although the percentage of larvae, pupae, and imagoes differs at times significantly from one period to another from the mean for the total 600 days, there appears to be no consistent pattern in these deviations as regards total population size. In sum, it cannot be concluded that percentage composition is related closely to total population density.

The data of Table 1 and Figure 1 are based on means computed by 30-day periods. Thus, for example, the mean population size at 300 days is a mean of six separate cultures. To the reader familiar with population phenomena it is obvious that, although 300 days appears as the low in Figure 1, it is not necessarily the low for every one of the six cultures. This fact is dealt with in Table 2, where the lowest density is chosen arbitrarily from each mating irrespective of the age of the culture and 210 days laid off on either side of that point. Table 2 shows the mean low density to occur at the mean age of 295 days. The table also shows how one culture varies from another. It is apparent that all six populations possess an oscillation cycle and that the decline period tends to be more gradual than the growth period. These data are graphed in Figure 2 on a logarithmic ordinate and arithmetic abscissa. This figure gives a good smoothed picture of the form of the oscillation.

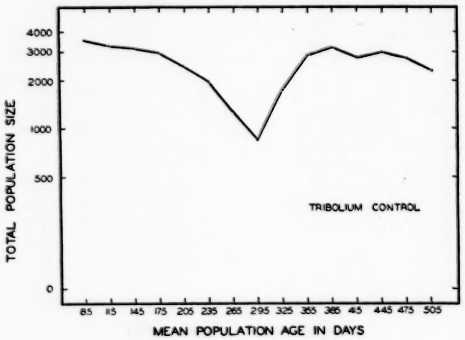


FIG. 2.—Population growth of *Tribolium* controls with low-density points set equal. Arith.-log. grid.

In this paper we shall describe by means of graphs the course of populations treated individually as well as averaged. Although this treatment adds to the bulk of the paper and perhaps may seem irrelevant to the reader, it has two virtues: first, it depicts immediately the interculture variability which in some instances is considerable, and, second, it presents the data in a form that is better suited for possible later analysis by students of theoretical populations. Figure 3 shows the *Tribolium* controls plotted as single cultures. This graph does not change any of the major conclusions just discussed. However, the comparison of the curves is instructive and should be made by the reader.

Bodenheimer (1938) devised a method of representing certain aspects of population dynamics that has some application here. He applied his schema primarily to populations of *Drosophila* and honeybees, although Chapman's (1928) data on *Tribolium* were also used to a lesser extent. Table 3 is a simplification of Bodenheimer's protocol based on the six *Tribolium* control cultures just discussed. In the table Columns A, B, and C are all obvious entries. Column D—the potential increase of the population—represents the maximum number of eggs the population could produce, assuming that there is a

consistent oviposition rate, an equal sex ratio, and nothing in the environment to reduce this fecundity. This index is obviously theoretical and arbitrary, but it has a certain interpretative value. Before it can be computed it is necessary to arrive at a standard and empirical expression of fecundity. A good deal of experience with the egg-laying

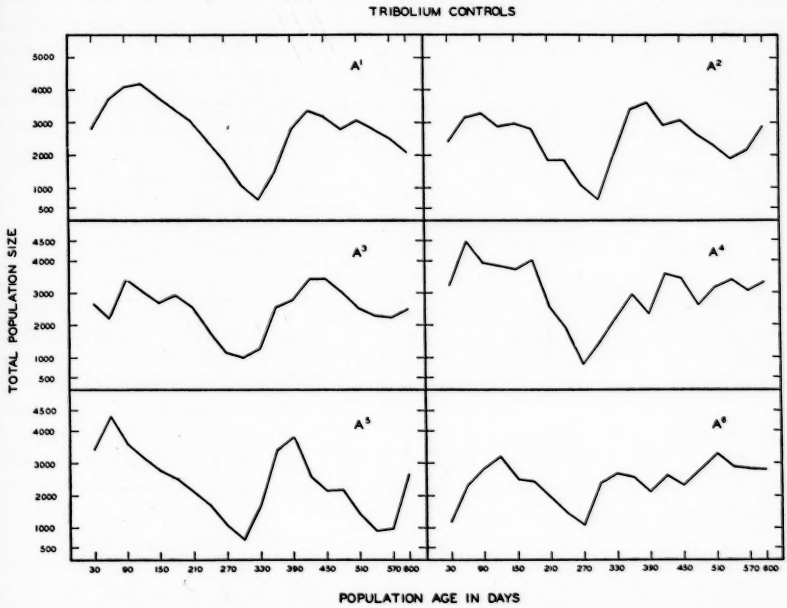


FIG. 3.—Population growth of *Tribolium* controls. Curves based on individual cultures

performance of *Tribolium* suggests that a suitable mean figure to use is 90 eggs per female per 30-day period. Thus the potential increase equals

$$90 \times \tfrac{1}{2} \text{ (imagoes)} .$$

These are the computations appearing in Column D. Column E is an expression of population growth rate per past 30-day period. It is computed as follows:

$$E = \frac{\text{Total population}}{\text{Total population at last 30 days}} .$$

This index merely puts in ratio form the change in total population size. If the quotient is greater than 1, the culture is growing; if less than 1, the culture is declining. Column F —the intensity of the struggle for existence—is computed as follows:

$$F = \frac{\text{Potential increase}}{\text{Rate of growth}} = \frac{D}{E} .$$

This index is again a quotient and gives in Bodenheimer's opinion a numerical approximation of the stringency of competition. We are not too convinced of the usefulness of this index, in part because we have not followed the logic behind it completely and in part because Bodenheimer does not himself evaluate it critically in his book. We include it in our tables primarily because it has been used by others and may be developed in greater detail in the future.

TABLE 3

Tribolium CONTROLS ARRANGED IN BODENHEIMER'S CLASSIFICATION

(A) Age	(B) Mean Total Population	(C) Mean Number Imagoes	(D) Potential Increase of the Population per Past 30 Days	(E) Rate of Growth	(F) Intensity of the Struggle for Existence
30	2,629.2	90.8	4,086.0	.....	.....
60	3,387.8	1,551.0	69,795.0	1.28	54,527.3
90	3,500.0	1,524.2	68,589.0	1.03	66,591.3
120	3,398.5	1,382.5	62,208.0	0.97	64,131.9
150	3,092.3	1,226.3	55,179.0	0.91	60,636.3
180	2,870.0	997.0	44,865.0	0.93	48,241.9
210	2,361.3	792.8	35,676.0	0.82	43,507.3
240	1,870.2	567.2	25,524.0	0.79	32,308.9
270	1,368.5	404.0	18,180.0	0.73	24,904.2
300	1,191.3	315.3	14,184.0	0.87	16,303.4
330	1,791.8	331.7	23,922.0	1.50	15,948.0
360	2,754.0	614.8	27,666.0	1.54	17,964.9
390	2,944.2	694.3	31,239.0	1.07	29,195.3
420	3,101.8	699.8	31,491.0	1.05	29,991.4
450	3,009.8	648.3	29,169.0	0.97	30,071.1
480	2,702.0	639.0	28,755.0	0.90	31,950.0
510	2,056.3	572.2	25,749.0	0.95	27,104.2
540	2,320.8	552.3	24,849.0	0.87	28,562.1
570	2,320.7	494.0	22,230.0	0.99	22,454.5
600	2,692.3	677.5	30,483.0	1.16	26,278.4

An examination of Table 3 shows two tendencies. (1) The potential increase starts out low owing to the presence of few imagoes but at 60 days, after a second generation has emerged, rises many fold and maintains a high level during the 30–180-day period. The potential increase drops during the 210–330-day period when the populations are at the bottom of their cycle, and then it rises somewhat as the populations build up toward their second peak. However, it does not rise so high as in the early stages of population growth, since the imagoes do not increase to their earlier level. (2) The intensity of the struggle for existence follows essentially the same pattern. The aggregate competition between beetles is highest during the first period and then drops as the culture wanes. There is some build-up of this component during the third period, but it does not attain the force reached earlier. In the fourth period the intensity drops some-

what but not to the same degree as it did in the second period. On the basis of the observed dampened oscillations this is to be anticipated.

At this point let us ask the question: Why, in a paper dealing with interspecific competition, must so much time be spent on single-species cultures? The answer is, of course, that the latter furnish a control against which the experiments can be tested. In this investigation we must be able to say that the single species live indefinitely as cultured here and that their populations follow a certain growth form. If these two points are known, then deviations in the experiments from the control pattern can be evaluated. For example, if one species that grows well when alone becomes extinct when in association with another species, it can be concluded, other things being equal, that the first species is driven out by the pressure of competition.

Since it has been shown for the present controls that *Tribolium* has a major oscillation during its early growth, it becomes particularly important to have a clear picture of this cycle. The cycle is a property of *Tribolium* and is not due to association with *Gnathoceros* and *Trogoderma*. Nevertheless, it persists when *Tribolium* is living with other species and, as we shall see, frequently is of considerable importance in shaping the total population trend. As to the cause of the oscillations, more experimentation is needed before definite conclusions can be drawn. Some work along this line is in progress. There are some tentative data suggesting that infection of the beetles with a sporozoan, *Adelina* (see Park, 1934), may be responsible. In short, the cycle may represent a pandemic type of phenomenon. Speculation on this point must await addi-

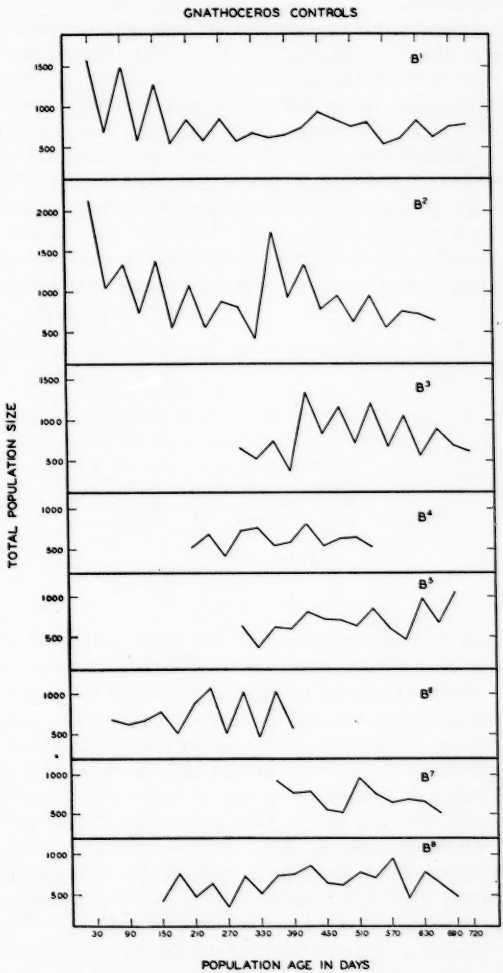


FIG. 4.—Population growth of *Gnathoceros* controls. Curves based on individual cultures. B^3 through B^8 have served as interspecific experimentals during their early history and are used as controls after their competitor becomes extinct.

tional evidence. Chapman (1933) has carried this analysis further than anyone else, and he is of the opinion that oscillations in *Tribolium* populations can be caused by disease infection.

Gnathoceros cornutus.—The data on *Gnathoceros* population growth are based on census observations made every 30 days over a 720-day span on eight different cultures.[3] The growth trend computed as a mean of these records is shown in Figure 1, the individual curves in Figure 4, and the data on percentage composition and total population size are summarized in Table 4. The graphs show an entirely different pattern for *Gnathoceros* than for *Tribolium* populations. The former curves are small but less variable and give no evidence of major oscillations. There are minor waves in the curve that suggest, with certain exceptions, that a large census tends to be followed the next 30 days by a smaller census. This "high-low" feature has not been analyzed. It may represent merely a life-cycle phenomenon, i.e., a wave of emergences and subsequent reproduction about every 60 days. Or it may be associated with egg and pupa cannibalism. Probably

TABLE 4

COMPOSITION AND TOTAL SIZE OF *Gnathoceros* CONTROLS IN PERCENTAGE ($n = 129$)

	Mean	S.D.	C.V.
Larvae	83.4 ± 0.51	8.6 ± 0.36	10.3
Pupae	2.5 ± 0.17	2.9 ± 0.12	118.7
Male imagoes	6.3 ± 0.21	3.58 ± 0.15	56.6
Female imagoes	7.8 ± 0.21	3.5 ± 0.15	45.1
Total population	762.5 ± 16.2	273.3 ± 11.5	35.8

both factors are involved. The basic pattern is present in all the individual curves of Figure 4, although there is considerable difference as to the time of a high or a low count.

From the point of view of the experiments to follow, however, *Gnathoceros* can be thought of as maintaining its populations at a mean of about 762 (± 16.23) beetles per 100 gm. of medium. The standard deviation shows that two-thirds of the time the population counts do not exceed 1,035 beetles or fall below 489 beetles. The percentages of Table 4 show that the larvae constitute the bulk of the population (83 per cent) and vary little from that figure. The imagoes contribute, on the average, about 14 per cent to the total population, but they are much more variable in their distribution than the larvae. The pupae are sporadic in appearance. They may be present in some counts and completely absent in others. This is due to the short duration of the stage and to the fact that the pupae are heavily cannibalized.

Table 5 suggests that the sex ratio of *Gnathoceros* imagoes deviates from an expected 1:1 frequency. Since practically nothing is known about this point, the sex ratios in each of the eight populations were examined individually. These data appear in Table 5. There it is shown that in six of the eight cultures there are significantly more females than males. In one of the cultures the difference in favor of females is borderline ($P = 0.0918$), and in one the ratios do not deviate significantly from an expected frequency.

[3] At the time of analysis two of the cultures were 720 days old; two, 690 days; two, 660 days; one, 600 days; and one, 390 days.

TABLE 5

SEX RATIO ANALYSIS OF *Gnathoceros* POPULATIONS

Mating	Male Imagoes (Percentage)	Female Imagoes (Percentage)	Imagoes Examined	Probability that the Sex Ratios Differ from a 1:1 Expectation
B^1	45.3	54.7	2,146	<0.0000
B^2	50.3	49.7	2,670	>0.5000
B^3	44.0	56.0	1,244	<0.0000
B^4	43.9	56.1	2,334	<0.0000
B^5	46.9	53.1	731	0.0918
B^6	39.7	60.3	1,496	<0.0000
B^7	42.9	57.1	1,289	<0.0000
B^8	40.8	59.2	882	<0.0000
Mean...	44.8	55.2	12,792	<0.0000

TABLE 6

Gnathoceros CONTROLS ARRANGED IN BODENHEIMER'S CLASSIFICATION

(A) Age	(B) Mean Total Population	(C) Mean Imagoes		(D) Potential Increase of the Population per Past 30 Days	(E) Rate of Growth	(F) Intensity of the Struggle for Existence
		Males	Females			
30	1,855.0	42.5	38.5	2,310.0	.....	.....
60	812.0	44.3	36.3	2,178.0	0.44	4,950.0
90	1,148.0	56.0	51.3	3,078.0	1.41	2,183.0
120	666.7	47.7	46.3	2,778.0	0.58	4,789.0
150	936.5	61.2	62.3	3,738.0	1.40	2,670.0
180	592.5	46.2	58.3	3,498.0	0.63	5,552.4
210	825.7	56.0	64.5	3,870.0	1.39	2,784.2
240	713.8	65.5	64.7	3,882.0	0.86	4,514.0
270	622.8	57.0	60.6	3,636.0	0.87	4,179.3
300	732.4	53.7	66.8	4,008.0	1.17	3,425.6
330	488.3	54.7	58.6	3,516.0	0.67	5,247.8
360	891.7	49.4	58.1	3,486.0	1.83	1,904.9
390	678.9	38.6	55.8	3,348.0	0.76	4,405.3
420	915.4	37.7	50.7	3,042.0	1.35	2,253.3
450	726.3	39.7	52.3	3,138.0	0.79	3,972.2
480	806.7	40.4	52.8	3,168.0	1.11	2,854.1
510	720.3	42.0	53.8	3,228.0	0.89	3,627.0
540	840.4	40.9	52.7	3,162.0	1.17	2,702.6
570	657.6	34.1	49.7	2,982.0	0.78	3,823.1
600	655.1	33.4	51.7	3,102.0	0.99	3,133.3
630	762.7	37.2	56.2	3,372.0	1.16	2,906.9
660	658.5	38.7	51.7	3,102.0	0.86	3,607.0
690	745.3	37.3	47.7	2,862.0	1.13	2,532.7
720	700.5	31.0	55.0	3,300.0	0.94	3,510.6

The mean of all eight populations shows that the females are decidedly more numerous than the males. Thus it appears that a real sex differential exists for *Gnathoceros* in most of these cultures. The difference cannot be attributed to faulty recognition, since the males are distinguished readily from females on the basis of their great mandibular development. Whether the difference represents a sex-linked lethal effect or some environmental selection remains to be explored.

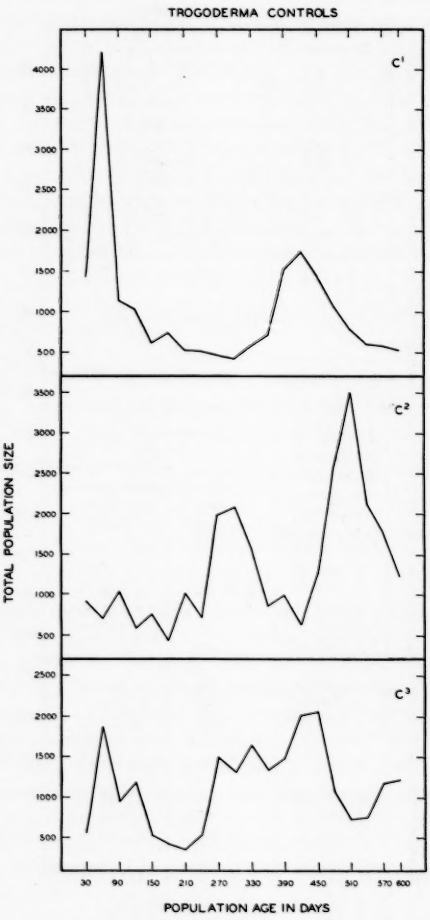


FIG. 5.—Population growth of *Trogoderma* controls. Curves based on individual cultures.

In Table 6 the *Gnathoceros* data are tabulated according to Bodenheimer's method. On the basis of original fecundity assays (unpublished) it was determined that *Gnathoceros* females produce on the average about 60 eggs per 30-day period. This figure is used in calculating Column D. Since the number of females is known, the potential increase per 30-day period is sixty times the number of females. From Table 6 the following facts are apparent: (1) *Gnathoceros* is much less productive than *Tribolium*. This shows in the total population size, the number of imagoes, the potential increase, and the intensity of the struggle for existence. (2) So far as the struggle for existence is a criterion, the total competitive pressure within a *Gnathoceros* population is less than it is for *Tribolium*. Obviously, this does not mean that an individual *Tribolium* contributes more to this pressure than an individual *Gnathoceros*. It means only that the *Tribolium* build up heavier cultures. (3) The rate of growth is a good index of the "high-low" characteristic mentioned earlier. In Column E there are twenty instances where a high rate of growth is followed by a low rate (or conversely) and only two instances where such is not the case.

Trogoderma versicolor.—The data on *Trogoderma* population growth are based on census observations made every 30 days over a 600-day span on three different cultures. The growth trend computed as a mean of these records is shown in Figure 1, and the individual curves appear in Figure 5. It is apparent that the curve for the grouped *Trogoderma* cultures has a different pattern from that of *Gnathoceros* and a somewhat less different pattern than *Tribolium*. As we have seen, the *Tribolium* curve oscillates over long periods, and the *Gnathoceros* curve maintains itself close to an average value. *Trogoderma*

is much more variable than either of the other two species. In C^1 and C^3 it starts out low, rises quickly to a maximum, and then fluctuates erratically around a mean. The C^2 culture does not exhibit an initial spurt but builds up later. Clearly, some of these fluctuations are due to the small size of the sample, and some may be due to the technical error of losing certain eggs in the sifting process. It seems likely, however, that the major factor concerns the beetles' reproduction. Dick (1937) classified the oviposition of Coleoptera into four groups: Types "I, II, III and IV." Types I and II fit the species used in these investigations and are defined by Dick (p. 765) as follows:

In Type I ". . . . belong species which live only a short time as adults and lay all their eggs within a few days." Examples, *Bruchus obtectus*, *Lasioderma serricorne*, *Trogoderma versicolor*.

In Type II ". . . . is represented by species which live for a long time—often over a year—as adults, and produce eggs continuously over a long time." Examples, *Gnathoceros cornutus*, *Tribolium* sp., *Necrobia rufipes*.

It seems apparent that *Trogoderma* populations have a different growth form than *Tribolium* or *Gnathoceros*, largely owing to the oviposition pattern and short imaginal life. In Figure 1 at the 30-day count the total population is a function of the original 88 imagoes only. The latter are all dead at 30 days. Norris (1936) has shown that the mean duration of life of males is about 19 days and of females about 11 days. Our own observations based on smaller numbers are somewhat different.[4] Between the 30- and 60-day reading a second brood of imagoes typically emerges and then reproduces. This accounts for the rise at the time of the second census noticeable in C^1 and C^2. At 90 days the imago number is down again, and the population is declining. This decline continues with variations until, between the 240- and 270-day census, another sizable stock of imagoes gets established and greater reproduction ensues. From then on, alternation of imagoes with larvae and eggs gets "out of step," and the cultures maintain themselves at a higher level. Even so, there remains a sporadic character to the growth that is not found in *Tribolium* or *Gnathoceros*. We cannot suggest that all *Trogoderma* populations follow the precise form shown in Figure 1. The curves of Figure 5 show that this would be an erroneous assumption. Differences in handling, in stocks, environmental control, and coincidence all affect the curve. However, it is important to correlate the explosive nature of the growth with the reproductive characteristics of the imagoes. This is sound procedure in any population analysis.

The data on total population size and composition are presented in Table 7. The mean *Trogoderma* population over 600 days is 1,362 beetles per 100 gm. of medium. In two-thirds of the instances this figure does not go above 2,309 or below 415 beetles. As would be anticipated from the above discussion, this is a greater relative variation in population size than that exhibited by the two Tenebrionid species. This point is supported by the

[4] We have obtained the following mean life-duration figures for *Trogoderma versicolor* imagoes kept in whole-wheat flour (sifted through an 82-mesh sieve) plus 2 per cent yeast:

Series	Life-Duration (Days)		*n*
	Males	Females	
Grouped (14 pairs in 20 gm. medium)	7.7	11.1	14
Isolated (1 imago per 2 gm. medium)	11.0	16.2	30 (males) 37 (females)

coefficients of variability, which are 39 per cent for *Tribolium*, 35 per cent for *Gnathoceros*, and 69 per cent for *Trogoderma*. Table 7 shows that the larvae constitute about 90 per cent of the population, with the imagoes next and the pupae last.

In Table 7 there is a suggestion that *Trogoderma* males appeared more frequently in the counts than did *Trogoderma* females. This is tested in Table 8, where it turns out that in each of the three populations the difference is greater than could be accounted for on chance alone. Here, then, is a sex-ratio distortion in the opposite direction from that presented for *Gnathoceros*. Again, the causal factors are not known. Since male

TABLE 7

COMPOSITION AND TOTAL SIZE OF *Trogoderma* CONTROLS IN PERCENTAGE

($n=68$)

	Mean	S.D.	C.V.
Larvae	89.4± 0.98	12.0± 0.69	13.4
Pupae	1.8± 0.18	2.2± 0.13	122.9
Male imagoes	5.5± 0.33	4.1± 0.23	73.7
Female imagoes	3.3± 0.20	2.5± 0.14	76.2
Total population	1361.9±77.4	946.7±54.0	69.5

TABLE 8

SEX RATIO ANALYSIS OF *Trogoderma* POPULATIONS

Population	Male Imagoes (Percentage)	Female Imagoes (Percentage)	Imagoes Examined	Probability that the Sex Ratios Differ from a 1:1 Expectation
C^1	58.9	41.1	3,221	0.0000
C^2	64.1	35.9	4,259	0.0000
C^3	60.3	39.7	1,764	0.0000
Total	61.4	38.6	10,407	0.0000

Trogoderma can be distinguished from females only because they are smaller, it was decided to test the validity of this criterion. One hundred *Trogoderma* imagoes were removed at random from a stock culture. These beetles were sexed independently by the three authors. Then the beetles were killed, their sex determined by dissection (47 males; 53 females), and compared with the results made on the basis of body size. The agreement between all three authors and the actual sex ratio was perfect. From these results it is apparent that sex was determined with complete accuracy by size differentials. Thus, although there may be an occasional negligible error, the method of sexing used in these experiments is reliable.

Table 9 arranges the *Trogoderma* data in the form suggested by Bodenheimer. There was some difficulty in arriving at the best way to express potential increase, since the imagoes live less than 30 days. Finally it was decided to take the mean number of fe-

males per 30-day interval and multiply this figure by 122 eggs. The latter is based on Norris' (1936) report that *Trogoderma* produced 122 eggs per 11 days, which is the mean length of life of females. To illustrate by an example, suppose we wish to compute the potential increase that exists between the 240- and the 270-day reading. The mean number of females that lived over this period is 23.85 (i.e., 7.0 + 40.7 ÷ 2). Thus 23.85 × 122 = 2,910 eggs. The potential-increase and struggle-for-existence columns of Table 9 bring out the erratic character of *Trogoderma*'s population dynamics. Both of these in-

TABLE 9

Trogoderma CONTROLS ARRANGED IN BODENHEIMER'S CLASSIFICATION

(A) Age	(B) Mean Total Population	(C) Mean Imagoes		(D) Potential Increase of the Population per Past 30 Days	(E) Rate of Growth	(F) Intensity of the Struggle for Existence
		Males	Females			
30.....	967.3	0.0	0.0	.....	.....	.....
60.....	2,387.7	151.3	100.3	6,118	2.47	2,476.9
90.....	1,114.0	60.7	25.7	7,686	0.47	16,353.2
120.....	956.7	19.3	13.0	2,361	0.86	2,745.3
150.....	628.0	5.0	5.3	1,116	0.66	1,690.9
180.....	546.7	9.0	4.0	567	0.87	651.7
210.....	650.3	11.7	7.3	689	1.19	579.0
240.....	594.3	32.0	7.0	872	0.91	958.2
270.....	1,378.0	61.7	40.7	2,910	2.32	1,254.3
300.....	1,356.7	113.0	41.0	4,984	0.98	5,085.7
330.....	1,396.7	94.0	68.7	6,692	1.03	6,497.1
360.....	1,020.7	63.0	26.4	5,801	0.73	7,946.6
390.....	1,491.3	136.7	105.7	8,058	1.46	5,519.2
420.....	1,571.3	102.7	65.0	10,413	1.05	9,917.1
450.....	1,702.0	132.0	69.0	8,174	1.08	7,568.5
480.....	1,758.7	134.3	89.0	9,638	1.03	9,357.3
510.....	1,983.3	203.3	152.0	1,470	1.13	1,300.9
540.....	1,238.3	120.0	64.7	13,219	0.62	21,321.0
570.....	1,363.7	138.3	72.0	8,339	1.10	7,580.9
600.....	1,088.0	99.7	62.3	8,192	0.80	10,240.0

dices build up rapidly, sink to an early low point when the imagoes decline, and then build up as the population matures.

Comparison of Tribolium, Gnathoceros, and Trogoderma controls.—Before proceeding to the interspecific experiments, the data for the three species growing as single-species cultures can be summarized as follows:

1. Growth trends (Figs. 1, 3, 4, and 5).
   a) *Tribolium* exhibits a relatively smooth cyclic oscillation during its first 420 days of husbandry. Thereafter, there is some evidence of a dampened oscillation.
   b) *Gnathoceros*, after an initial spurt, settles down to an average value and maintains itself closely about that average.
   c) *Trogoderma* grows erratically. There is a high spurt followed by a low region and then a higher period of great variability.
   d) All three species grow successfully (i.e., do not die out) under the conditions of husbandry described.

2. Total size (Tables 1, 4, and 7). On the average, *Tribolium* maintains the largest cultures (2,538.5 ± 61.4 beetles per gm. medium); *Trogoderma* comes next (1,361.9 ± 77.4 beetles); and *Gnathoceros* populations are smallest (762.5 ± 16.23 beetles). As regards variability of census counts, *Trogoderma* is the most variable, *Tribolium* next, and *Gnathoceros* least.
3. Composition (Tables 1, 4, and 7; Fig. 6).
   a) In all three species the larvae constitute the bulk of the population (65 per cent for *Tribolium;* 83 per cent for *Gnathoceros;* and 89 per cent for *Trogoderma*).
   b) The relative frequency of imagoes is highest for *Tribolium* (27.3 per cent), next for *Gnathoceros* (14 per cent), and lowest for *Trogoderma* (8.8 per cent).
   c) The pupae are always relatively sparse and may be completely absent from any one count. The mean per cents are for *Tribolium* 7.2, for *Gnathoceros* 2.5, and for *Trogoderma* 1.8.
   d) The variability is indexed by the standard deviations and coefficients of variability tabulated in Tables 1, 4, and 7. These parameters should be studied.

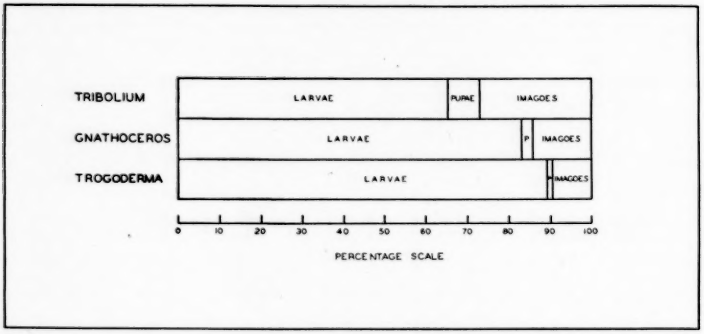


FIG. 6.—Percentage composition of control populations of *Tribolium*, *Gnathoceros*, and *Trogoderma*

   e) As to imaginal sex ratio, *Tribolium* does not deviate significantly from a 1 : 1 expectation; *Gnathoceros* produces on the average 44.8 males to 55.2 females, and *Trogoderma* produces 61.4 males to 38.6 females. The latter two deviations are significant statistically.

THE INTERSPECIFIC POPULATIONS

Tribolium and Gnathoceros.—The data on which the *Tribolium-Gnathoceros* interactions are based consist of (1) three matings (D^1, D^2, D^3) in which both species were started in equal density; (2) one mating (G) in which the initial density was 76 *Tribolium* to 12 *Gnathoceros*, and (3) one mating (I) in which the initial density was 12 *Tribolium* to 76 *Gnathoceros*. Since the *Tribolium-Gnathoceros* data are the most complicated, as well as the most interesting in many ways, we shall discuss them in some detail. The data are summarized in Tables 10 (D^1), 11 (mean of D^2 and D^3), and 12 (G, I) and graphed in Figures 7, 8, and 9. As an index of growth changes from one census to the next a simple ratio column appears in all the tables. In the present matings this index is computed always by dividing the total number of *Tribolium* for any particular census by the total number of *Gnathoceros*. Thus the index starts with a quotient value of 1 (44/44) in D; a value of 6.34 (76/12) in G; and a value of 0.16 (12/76) in I. The quotient is easily interpreted. As it increases in magnitude, *Tribolium* is besting *Gnathoceros;* as it decreases, *Gnathoceros* is driving out *Tribolium*.

First let us consider the D-matings. Figure 7 shows that two types of end results

take place despite the fact that the experiments are all started in the same way. In graph D^1 *Tribolium* and *Gnathoceros* are about even in numbers at 30 days. Thereafter, *Tribolium* rises steadily and reaches, between 120 and 210 days, its first high point.

TABLE 10

Gnathoceros-Tribolium COMPETITION IN THE D^1-MATING

Age	*Tribolium*	*Gnathoceros*	Ratio $\frac{T}{G}$
1	44	44	1.00
30	1,319	1,176	1.12
60	974	404	2.34
90	2,299	158	14.55
120	3,002	51	58.86
150	2,775	25	111.00
180	2,947	5	589.00
210	2,970	1	2,970.00
240	2,465	0	.....
270	2,030	.....	.....
300	1,641	.....	.....
330	4,133	.....	.....

TABLE 11

Gnathoceros-Tribolium COMPETITION: MEAN OF THE D^2-D^3–MATINGS (COMPUTED FOR ALL STAGES)

Age	Larvae		Ratio $\frac{T}{G}$	Pupae		Ratio $\frac{T}{G}$	Imagoes		Ratio $\frac{T}{G}$	Total		Ratio $\frac{T}{G}$
	Trib.	*Gnath.*		*Trib.*	*Gnath.*		*Trib.*	*Gnath.*		*Trib.*	*Gnath.*	
1	.....	.....	.....	.....	.....	.....	44.0	44.0	1.00	44.0	44.0	1.00
30	1,226.0	1,046.5	1.17	413.5	0.0	.....	62.5	37.5	1.67	1,702.0	1084.0	1.57
60	1,550.5	190.0	8.16	81.5	28.0	2.91	325.5	36.5	8.92	1,957.5	254.5	7.69
90	1,828.5	196.5	9.30	152.0	5.5	27.63	428.5	42.0	10.20	2,409.0	244.0	9.87
120	1,529.5	163.0	9.38	60.5	10.0	6.05	380.5	29.0	13.12	1,970.5	202.0	9.75
150	1,394.5	116.5	11.97	52.5	1.0	52.50	320.0	20.5	15.61	1,767.0	138.0	12.80
180	1,727.0	86.5	19.97	44.0	1.5	29.33	249.5	25.0	9.98	2,020.5	113.0	17.88
210	597.0	176.0	3.39	31.0	8.5	3.64	203.0	39.0	5.20	831.0	223.5	3.72
240	339.5	348.5	0.97	26.5	13.5	1.96	145.0	36.0	4.03	511.0	398.0	1.28
270	104.0	398.5	0.27	2.0	11.5	0.17	87.5	44.0	1.99	193.5	444.0	0.43
300	19.0	613.5	0.03	14.5	4.0	3.62	60.5	56.5	1.07	94.0	674.0	0.14
330	1.0	394.0	0.003	1.0	17.0	0.06	34.0	58.5	0.58	36.0	469.5	0.08
360	2.0	662.5	0.003	0.0	13.5	.....	29.0	66.0	0.44	31.0	742.0	0.04
390	0.5	502.0	0.001	0.0	18.5	.....	22.5	57.5	0.39	23.0	578.0	0.03
420	0.0	870.5	.....	0.0	12.5	.....	15.0	64.5	0.23	15.0	947.5	0.01
450	0.0	649.5	.....	0.0	8.0	.....	12.0	58.5	0.21	12.0	716.0	0.01
480	0.0	883.0	.....	0.0	4.0	.....	6.0	91.5	0.07	6.0	978.5	0.006
510	0.0	554.0	.....	0.0	4.0	.....	3.5	81.5	0.04	3.5	639.5	0.005
540	0.0	801.5	.....	0.0	11.5	.....	3.0	102.5	0.03	3.0	915.5	0.003
570	0.0	550.5	.....	0.0	10.5	.....	1.5	97.0	0.01	1.5	658.0	0.002
600	0.0	684.5	.....	0.0	7.0	.....	0.0	111.5	.....	0.0	803.0	.....

During this time *Gnathoceros* is consistently going down. By 240 days all *Gnathoceros* are gone, and the culture is now a pure *Tribolium* population. Since *Gnathoceros* controls maintain themselves indefinitely and since nothing has been altered except the introduc-

tion of *Tribolium*, we must conclude that in the D^1-mating the latter species drives out *Gnathoceros* with efficiency and dispatch. A glance at Figure 7 and Table 10 shows that *Tribolium* goes on in typical control fashion with a drop at 300 days and a sharp rise at 330 days. In short, the 210-day association with *Gnathoceros* does not affect the subsequent course of the population.

Figure 7, graphing the mean[5] of D^2 and D^3, and Figure 8, depicting these cultures individually, present a different picture in the sense of the end result. Yet the interpretation seems consistent with that discussed above. In both D^2 and D^3, *Tribolium* rapidly

TABLE 12

Gnathoceros-Tribolium COMPETITION FOR THE G^1- AND I^1-MATINGS

Age	G^1-Mating			I^1-Mating		
	Tribolium	*Gnathoceros*	Ratio $\frac{T}{G}$	*Tribolium*	*Gnathoceros*	Ratio $\frac{T}{G}$
1	76	12	6.34	12	76	0.16
30	2,695	366	7.36	316	1,406	0.22
60	2,264	162	13.97	82	732	0.11
90	3,374	21	160.00	935	664	1.41
120	3,010	7	430.00	434	386	1.12
150	2,745	10	274.00	1,738	392	4.43
180	2,925	6	487.00	2,027	301	6.73
210	2,596	3	865.00	1,260	165	7.64
240	1,812	1	1,812.00	2,436	110	2.21
270	1,149	1	.....	1,224	185	6.67
300	1,021	1	.....	521	250	2.08
330	1,243	0	.....	277	603	0.46
360	2,520	.....	.....	102	925	0.11
390	2,890	.....	.....	49	771	0.06
420	3,456	.....	.....	49	783	0.06
450	.....	.....	.....	17	563	0.03
480	.....	.....	.....	13	527	0.02
510	.....	.....	.....	10	951	0.01
540	.....	.....	.....	6	754	0.008
570	.....	.....	.....	6	651	0.009
600	.....	.....	.....	4	693	0.006
630	.....	.....	.....	3	677	0.004
660	.....	.....	.....	3	507	0.005

gets ahead of *Gnathoceros* and reaches a peak that extends between 90 and 180 days. During this time *Gnathoceros* is steadily declining. This decline is a repetition of the events of D^1. The reason that D^2 and D^3 differ from D^1 seems to be due to the following two factors, both of which pertain to *Tribolium* growth only: (1) The *Tribolium* populations of D^2 and D^3 do not reach so great a density over the 90–180-day intervals as did D^1 (means = 2,041 beetles for D^2, D^3; 2,756 beetles for D^1), and (2) the decline in *Tribolium* comes earlier in the D^2, D^3 than in the D^1 culture. In Table 11 note that, as *Tribolium* starts to decline owing to some factor not connected with its competitor, *Gnathoceros* immediately starts to build up. The total-population column at 180 days shows *Tribolium* has a count of 2,020 and *Gnathoceros* 113. The latter figure is the lowest

[5] In certain cases replicated experiments have been grouped and averaged. This has been done only in those instances where the experiments are basically similar in trend and end result.

that *Gnathoceros* reaches. Between 180 and 210 days *Tribolium* drops to 831 beetles. During the same interval *Gnathoceros* increases from 113 to 223! In a control culture *Tribolium* would build up again. Instead it goes down steadily and finally becomes extinct at 600 days. *Gnathoceros* rapidly attains control size and thereafter displays the short high-low trend characteristic of its growth. In sum, it appears that there is no real distinction in terms of causes between D^1 and D^2–D^3. In both cases the end results depend on what happens to *Tribolium*. *Tribolium* is not affected by *Gnathoceros* until it reaches a density that is abnormally low. If it gets to this low density, then *Gnathoceros* becomes a successful competitor, and the flour beetle is slowly driven out. These D-experiments suggest that the initial density is not necessarily the important factor in controlling the future of the population. It is the numerical "establishment" of *Tribolium* that is significant.

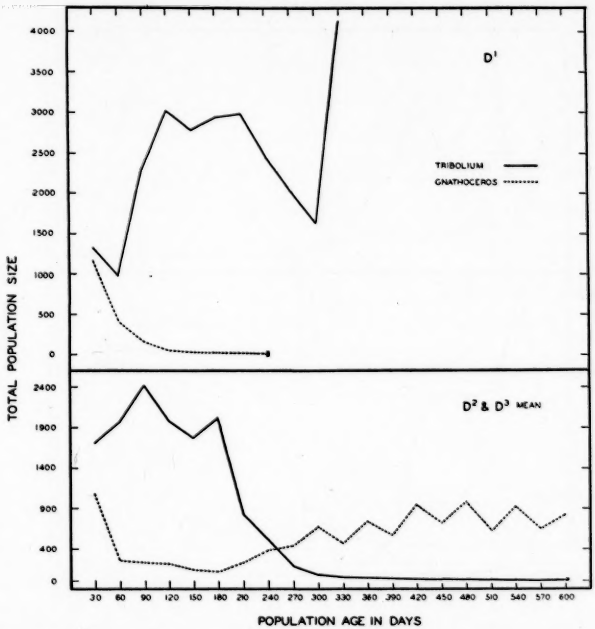


FIG. 7.—Population growth in the *Tribolium-Gnathoceros* cultures. Upper graph, D^1; lower graph, mean of D^2 and D^3.

The points just made for the D-matings are supported by the G- and I-matings graphed in Figure 9 and summarized in Table 12. Let us consider first the G case where the imagoes were introduced in the ratio of 76 *Tribolium* to 12 *Gnathoceros*. Here the data are clear cut. The *Tribolium*, as a consequence of their superior initial density, immediately establish themselves and build up a large population by the first census (2,695 beetles). The mean number of *Tribolium* over the 90–180-day interval is 3,013. The *Gnathoceros*, having made a feeble start at best (366 beetles at 30 days), are competing with a vigorous *Tribolium* population. The result is that they immediately and rapidly

start to decline and are extinct by the time *Tribolium* reaches its low point. In this case there is never an opportunity for successful competition on the part of *Gnathoceros;* they are bested from the start.

In the I-mating *Gnathoceros* was started high and *Tribolium* low. The drop in *Gnathoceros* during the 30–60-day interval is characteristic of the controls and cannot be considered as primarily due to *Tribolium*. However, after 60 days *Tribolium* begins to build up. The imagoes increase from the start of the experiment up to the 210-day count. This is important because they are the reproducing element in the population and they do not seem to be restrained by their competitor. Apparently, *Gnathoceros* is not a successful

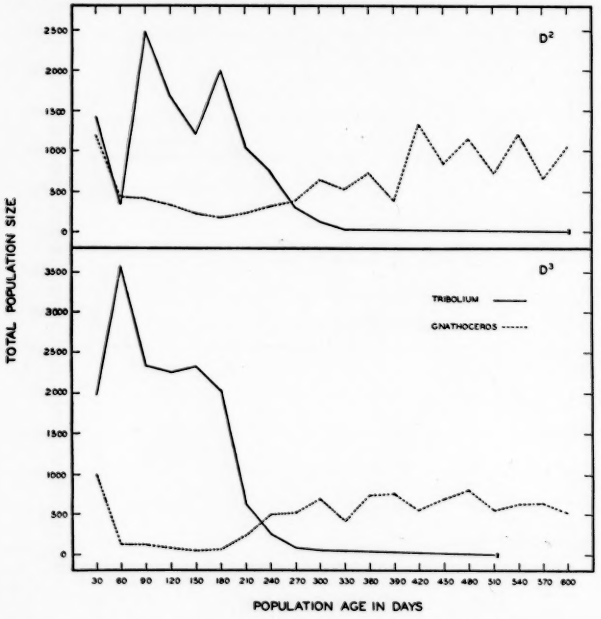


FIG. 8.—Population growth in the *Tribolium-Gnathoceros* cultures. Upper graph, D^2; lower graph, D^3

competitor of *Tribolium* during the early stage of the culture, when, presumably, the latter is not hindered by infection. As the *Tribolium* density increases, *Gnathoceros* goes down. This is the experience of the other matings. However, *Tribolium* has not established itself well enough during its first 240 days of life completely to drive out *Gnathoceros*, although the latter has fallen from 732 at the 60-day census to 110 at the 240-day census. At 240 days *Tribolium* starts its decline. The total density drops from 2,436 beetles to 1,224 at 270 days, to 521 at 300 days, to 49 (46 imagoes) at 390 days. *Gnathoceros* starts to rise between 240 and 270 days and attains its control density around 330 days. Thereafter, *Tribolium* goes slowly down until at 660 days only 3 imagoes remain. Between the 660- and 690-day reading something took place in the culture that complicates the picture. At the 690 count there were 26 *Tribolium* larvae, 7 pupae, and 7 imagoes. In other words, the *Tribolium* population had increased even in the face of *Gnatho-*

ceros pressure. Whether this increase represents a contamination (i.e., inadvertent entry of *Tribolium* from another source) or a real increase cannot be said. At the time of this writing the I-mating has been observed through the 750-day count, and the *Tribolium* have increased slightly. The culture will be continued until a definite conclusion can be drawn. That conclusion will be reported in the body of other experimental analyses.

It is interesting to note for matings D^2, D^3, and I, where *Tribolium* is bested by *Gnathoceros*, that it is the larvae and pupae that are driven out in greatest proportion and not the imagoes. The latter, although highly vulnerable to the supposed parasitic infection,

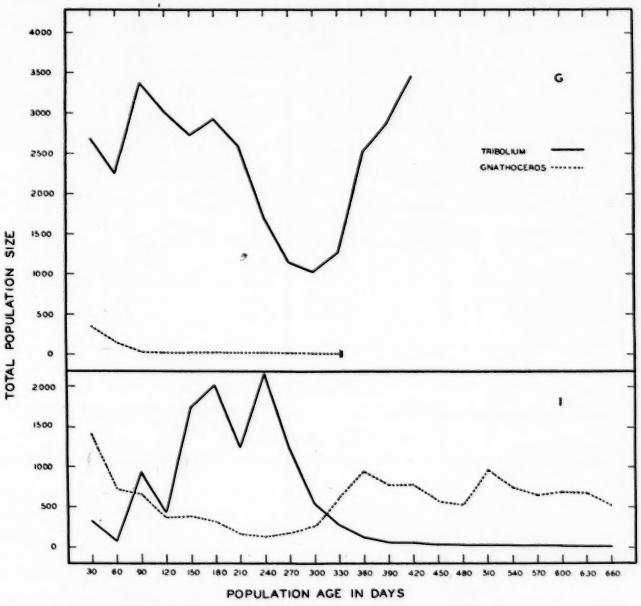


FIG. 9.—Population growth in the *Tribolium-Gnathoceros* cultures. Upper graph, G; lower graph, I

apparently are relatively immune to the pressure of *Gnathoceros* competition. They constitute the small number that lingers on. Finally they die, presumably of old age. This suggests that *Gnathoceros* may function as a predator on *Tribolium* eggs, small larvae, and pupae. This predation becomes significant only when *Tribolium* imagoes die off and thus reduce the reproductive capacity of the population. The nature of the competition pressure exerted by *Tribolium* on *Gnathoceros* is not clear, but in part it may be a predation effect also.

Recapitulation.—The most important points emerging from this experimental study of *Gnathoceros* and *Tribolium* in population association may be stated as follows:

1. If *Tribolium* gets well established as a total population, it is not affected dangerously by *Gnathoceros*. By "dangerously" we mean extinction or near-extinction, and we stress that point since there is some suggestion that *Tribolium* populations do not get so large when in association with *Gnathoceros* as they do otherwise.

2. A well-established *Tribolium* culture always drives out *Gnathoceros*.
3. If *Tribolium* is reduced to a certain point, it is driven out by *Gnathoceros*. The latter presumably have their competitive effect primarily on the eggs, larvae, and pupae.

TABLE 13

Tribolium-Trogoderma COMPETITION: MEAN OF THE E^1-E^2–MATINGS (COMPUTED FOR ALL STAGES)

Age	Larvae		Ratio $\frac{Trib.}{Trog.}$	Pupae		Ratio $\frac{Trib.}{Trog.}$	Imagoes		Ratio $\frac{Trib.}{Trog.}$	Total		Ratio $\frac{Trib.}{Trog.}$
	Trib.	*Trog.*		*Trib.*	*Trog.*		*Trib.*	*Trog.*		*Trib.*	*Trog.*	
0–1	.....	.....	.....	.....	.....	.....	44	44	1.00	44	44	1.00
30	1,765	910	1.94	302	0	.....	40	0	.....	2,107	910	2.31
60	1,660	1,031	1.61	94	16	5.87	848	197	4.30	2,602	1,244	2.09
90	2,432	89	27.32	130	.....	.....	882	182	4.85	3,444	271	12.71
120	2,145	10	214.50	92	.....	.....	734	.....	.....	2,971	10	297.10
150	2,644	0	.....	65	.....	.....	674	.....	.....	3,383	.....	.....
180	2,180	.....	.....	26	.....	.....	603	.....	.....	2,809	.....	.....
210	1,935	.....	.....	144	.....	.....	512	.....	.....	2,591	.....	.....
240	1,836	.....	.....	147	.....	.....	380	.....	.....	2,363	.....	.....
270	988	.....	.....	205	.....	.....	349	.....	.....	1,542	.....	.....
300	1,560	.....	.....	342	.....	.....	446	.....	.....	2,348	.....	.....
330	2,257	.....	.....	265	.....	.....	462	.....	.....	2,984	.....	.....
360	2,673	.....	.....	337	.....	.....	738	.....	.....	3,748	.....	.....

TABLE 14

Tribolium-Trogoderma COMPETITION IN THE H^1-H^2–MATINGS (MEAN) AND THE K^1-MATINGS

Age	*Tribolium*	*Trogoderma*	Ratio $\frac{Trib.}{Trog.}$	*Tribolium*	*Trogoderma*	Ratio $\frac{Trib.}{Trog.}$
1	76	12	6.34	12	76	0.16
30	3,347	120	27.9	1,157	983	1.18
60	4,368	47	92.9	2,395	683	3.51
90	3,783	1	3783.0	2,723	51	53.4
120	3,510	1	3510.0	3,230	16	201.9
150	3,254	0	.....	2,512	9	279.1
180	2,784	.....	.....	2,448	4	612.0
210	2,359	.....	.....	1,990	2	995.0
240	1,817	.....	.....	1,465	2	732.5
270	939	.....	.....	1,314	1	1,314.0
300	1,060	.....	.....	2,216	0	.....
330	2,005	.....	.....	2,734	.....	.....
360	3,173	.....	.....	2,561	.....	.....

4. In the experiments reported here the major reduction of *Tribolium* populations is not due to *Gnathoceros* but to those same factors that cause the oscillations observed in control cultures.
5. If *Tribolium* populations maintained a high asymptotic density without large oscillations, it seems likely that *Gnathoceros* would always be driven out.

Tribolium and Trogoderma.—The data on which the *Tribolium-Trogoderma* interactions are based consist of (1) two matings (E^1 and E^2) in which both species were started in equal density; (2) two matings (H^1 and H^2) in which the initial density was 76 *Tribolium* to 12 *Trogoderma;* and (3) one mating (K) in which the initial density was 12 *Tribolium* to 76 *Trogoderma*. The data are summarized in Tables 13 (mean of E^1 and E^2) and 14 (mean of H^1 and H^2; K) and graphed in Figures 10 (E), 11 (H^1 and H^2; K) and 12 (H^1; H^2).

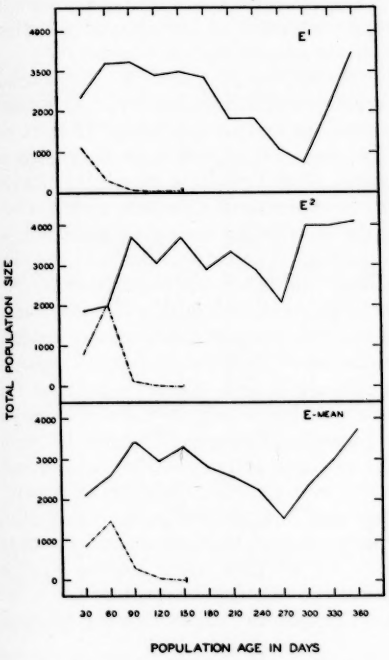


FIG. 10.—Population growth in the *Tribolium-Trogoderma* cultures. Upper graph, E^1; middle graph, E^2; lower graph, mean of E^1 and E^2. Solid line is *Tribolium*.

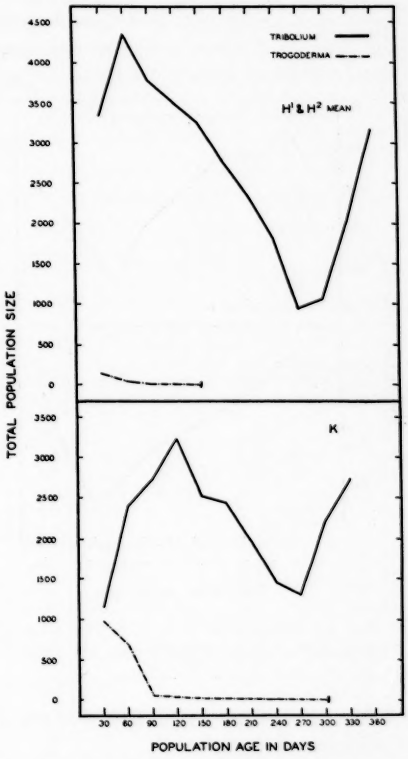


FIG. 11.—Population growth in the *Tribolium-Trogoderma* cultures. Upper graph, mean of H^1 and H^2; lower graph, K.

The major conclusion that emerges from these data is that *Tribolium* always drives out *Trogoderma*, irrespective of the initial-density conditions. Figure 10 and Table 13 show that, in the E-experiments, *Tribolium* starts out in normal fashion, reaches a mean population of 3,152 beetles between 90 and 180 days, and then declines. *Trogoderma* builds up during the first 60 days, and a new generation of imagoes emerges during the 30–60-

day interval. After 90 days all imagoes die out, and the last larvae disappear between the 120- and the 150-day interval. By 150 days the *Trogoderma* are extinct. Clearly, this extinction must be a function of some potent competitive pressure impinged on *Trogoderma* by *Tribolium*.

For the H-experiments, Table 14 and Figures 11 and 12 show that the *Tribolium* pressure is so rigorous that there is practically no effective reproduction on the part of the original imagoes and a second generation never emerges. Pragmatically speaking, the *Trogoderma* are extinct by 90 days, since only one larva in one culture lives during the 90–120-day interval. The H-experiments furnish an example of the drastic selection action of one species against another.

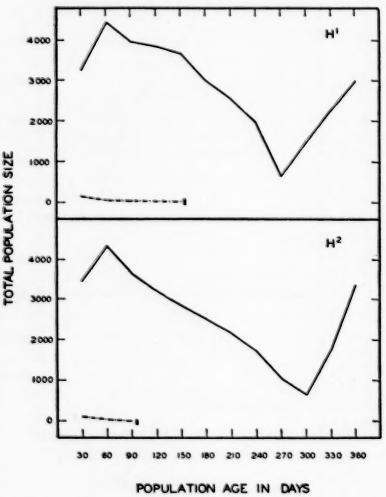


FIG. 12.—Population growth of *Tribolium-Trogoderma* cultures. Upper graph, H^1; lower graph, H^2. Solid line is *Tribolium*.

The K-experiment provides *Trogoderma* with a numerical advantage over *Tribolium* at the time the culture is started. In spite of this advantage *Trogoderma* is ineffectual as a competitor. The *Tribolium* population starts growing in characteristic fashion and reaches during the 90–180-day interval a mean size of 2,728 beetles. Thereafter, decline sets in. *Trogoderma* builds a total population of 983 beetles by 30 days, and during the 30–60-day interval the F_1 imagoes totaling 260 appear. In the absence of *Tribolium* pressure *Trogoderma* now should be in a favorable position for expansion. It has a balanced distribution of larvae, pupae, and imagoes. Instead, between 60 and 90 days, its population is reduced from a 683 to a 51 total. Only larvae remain; all pupae and imagoes are gone. The larvae are rapidly reduced between 90 and 120 days and then die off until they become extinct during the 270–300-day interval.

Summary.—The major points of the *Tribolium-Trogoderma* competition can be summarized as follows:

1. *Tribolium* always drives out *Trogoderma*.
2. The rate of extinction of *Trogoderma* is roughly proportional to the initial density of *Tribolium*.
3. *Trogoderma* produces an F_1 imago generation unless the early (0–60-day) pressure of *Tribolium* is exceedingly great. In the latter case (H), the only *Trogoderma* reproduction, is a product of the initial imagoes.
4. Once *Trogoderma* starts its decline, it goes out rapidly.
5. *Trogoderma* probably has some effect on the *Tribolium* populations. This point must await more specific analysis before definite statements are possible.

Gnathoceros and Trogoderma.—The data on which the *Gnathoceros-Trogoderma* interactions are based consist of (1) one mating (F) in which both species were started in equal density; (2) one mating (J) in which the initial density was 76 *Gnathoceros* to 12

Trogoderma; and (3) one mating (L) in which the initial density was 12 *Gnathoceros* to 76 *Trogoderma*. The data are summarized in Tables 15 (F) and 16 (J; L) and graphed in Figure 13.

In its most general form the *Gnathoceros-Trogoderma* competition can be summarized by pointing out that, of the two species, *Gnathoceros* is somewhat the stronger and usually, although not invariably, wins. The critical factor seems to be "establishment"—the degree to which one species gets an advantage over the other.

In mating F, where both species are started in equal density, *Gnathoceros* wins out after 750 days. It builds up its usual heavy larval population (1,174) during the first 30

TABLE 15

Gnathoceros-Trogoderma COMPETITION IN THE F-MATING (COMPUTED FOR ALL STAGES)

Age	Larvae		Ratio $\frac{G}{T}$	Pupae		Ratio $\frac{G}{T}$	Imagoes		Ratio $\frac{G}{T}$	Total		Ratio $\frac{G}{T}$
	Gnath.	*Trog.*		*Gnath.*	*Trog.*		*Gnath.*	*Trog.*		*Gnath.*	*Trog.*	
0–1	.....	.....	.....	.....	.....	.....	44	44	1.00	44	44	1.00
30	1,174	241	4.87	0	0	.....	38	0	.....	1,212	241	5.03
60	200	86	2.33	23	10	2.30	31	28	1.11	254	124	2.05
90	841	652	1.29	1	0	.....	92	23	4.00	934	675	1.38
120	198	413	0.48	16	2	8.00	104	51	2.04	318	466	0.68
150	263	463	0.57	1	0	.....	109	1	109.00	373	464	0.80
180	127	253	0.50	10	0	.....	86	6	14.33	223	259	0.86
210	146	201	0.73	4	0	.....	86	5	17.20	236	206	1.14
240	296	167	1.77	1	0	.....	66	0	.....	363	167	2.17
270	175	153	1.14	18	0	.....	76	1	76.00	269	154	1.76
300	540	147	3.67	1	0	.....	98	2	49.00	639	149	4.29
330	238	125	1.90	14	0	.....	122	0	.....	374	125	2.99
360	505	115	4.39	10	0	.....	109	0	.....	624	115	5.43
390	499	98	5.09	12	0	.....	106	0	.....	617	98	6.29
420	722	79	9.14	7	0	.....	79	0	.....	808	79	10.23
450	612	68	9.00	32	0	.....	97	1	97.00	741	69	10.74
480	669	61	10.97	8	0	.....	53	0	.....	730	61	11.97
510	528	53	9.96	23	0	.....	105	1	105.00	656	54	12.15
540	698	41	17.02	12	0	.....	90	1	90.00	800	41	19.51
570	541	23	23.52	11	0	.....	58	0	.....	610	23	26.52
600	381	16	23.81	19	0	.....	73	1	73.00	473	17	27.82
630	881	6	146.83	0	0	.....	102	0	.....	983	6	163.83
660	524	4	131.00	49	0	.....	103	0	.....	676	4	169.00
690	955	3	318.33	0	0	.....	94	0	.....	1,049	3	348.67
720	419	1	419.00	4	0	.....	259	0	.....	682	1	682.00

days of culture. For the same interval the *Trogoderma* population is low (241 larvae), the 44 seeded imagoes have died, and the F_1 generation has not yet appeared. Between 30 and 60 days both species drop; some *Trogoderma* imagoes emerge and oviposit, and *Gnathoceros* is beginning to pupate. At 90 days new reproduction has bolstered both species, and both are in a favorable position for further growth. At 120 days the two have dropped but still are about evenly matched. During the 120–50-day interval, however, *Gnathoceros* attains the telling start over *Trogoderma*—not in the sense that it builds up a population of much greater density but in the sense that *Trogoderma*, by losing its imagoes, thereby loses its reproducing power. From then on *Trogoderma* gradually but surely dwindles. It is not a fecund culture. *Gnathoceros* assumes its control

TABLE 16

Gnathoceros-Trogoderma COMPETITION IN THE J- AND L-MATINGS

AGE	J-MATING			L-MATING		
	Gnathoceros	*Trogoderma*	Ratio $\frac{G}{T}$	*Gnathoceros*	*Trogoderma*	Ratio $\frac{G}{T}$
1	76	12	6.34	12	76	0.16
30	1,551	63	24.62	602	555	1.08
60	558	24	23.25	148	2,004	0.07
90	659	94	7.01	181	989	0.18
120	184	24	7.67	63	1,183	0.05
150	419	17	26.65	44	508	0.09
180	753	9	83.67	56	441	0.13
210	488	6	81.33	53	371	0.14
240	645	5	129.00	29	537	0.05
270	366	5	73.20	20	1,539	0.01
300	714	4	178.50	7	1,336	0.005
330	512	4	128.00	3	1,895	0.001
360	728	4	182.00	0	1,432	.....
390	749	3	249.67	.....	1,607	.....
420	853	2	426.50	.....	.....	.....
450	636	1	636.00	.....	.....	.....
480	630	1	630.00	.....	.....	.....
510	775	0	.....	.....	.....	.....

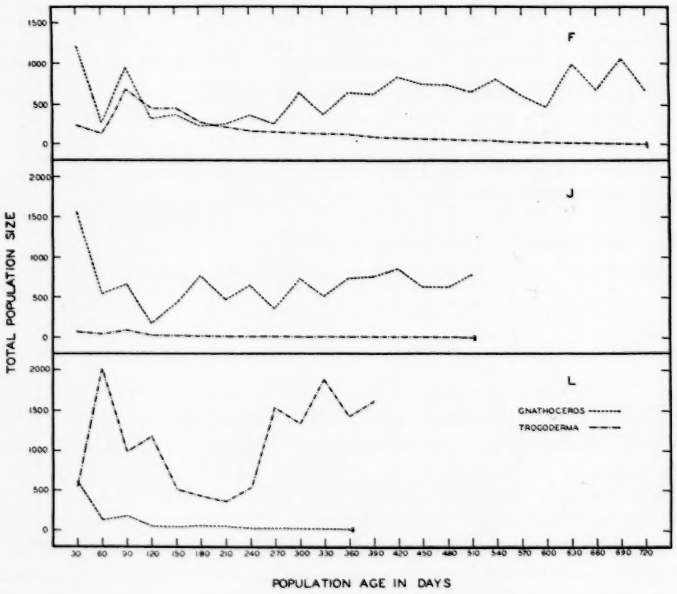


FIG. 13.—Population growth of *Gnathoceros-Trogoderma* cultures. Upper graph, F; middle graph, J; lower graph, L.

appearance by 300 days. For this case it thus appears that *Gnathoceros* competes successfully against *Trogoderma* by keeping the latter from building up an imaginal population.

In the J-mating *Gnathoceros* is started high and immediately gets well established. At 30 days there are 1,484 larvae and 67 of the original imagoes. Under this pressure *Trogoderma* fails to reproduce effectively. There is a feeble peak at 90 days due to the emergence of a few F_1 imagoes, but thereafter the population declines and becomes extinct by 480 days. In a sense *Trogoderma* is extinct at 90 days, for not one pupa or imago is produced during the 90–480-day interval. *Gnathoceros* assumes its control character by 150 days.

The L-experiment, although different in end result, correlates nicely with the above matings. By virtue of a high initial seeding, *Trogoderma* is successfully competing with *Gnathoceros* at 30 days and has built up a population of 555 larvae. During the 30–60-day interval 220 imagoes emerge. These reproduce with explosive force, and by 60 days a total population of 2,004 *Trogoderma* is counted. It is plain from the data that this high *Trogoderma* density is not without its effect on *Gnathoceros*, since the latter drop from 602 at 30 days to 148 at 90 days. *Trogoderma* is now well established, and its imagoes keep emerging and reproducing. In the face of this erratic pressure *Gnathoceros* dwindles, both as to larvae and as to adults, and by 360 days it is extinct.

Summary.—The major points of the *Gnathoceros-Trogoderma* competition can be summarized as follows:

1. *Gnathoceros* when started at equal or higher initial density drives out *Trogoderma*. The latter disappears faster in the J-mating than in the F.
2. *Trogoderma* seems to be driven out because it fails to keep enough imago generations emerging to reproduce itself. This suggests that *Gnathoceros* pressure in some way inhibits *Trogoderma*'s pupation and emergence.
3. *Trogoderma* can drive out *Gnathoceros*, if it gets well established. It seems particularly important that a large F_1 brood of imagoes emerges. The nature of the *Trogoderma* pressure on *Gnathoceros* is not clear.

The M-mating.—In addition to the two-species crosses, one mating was started with *Tribolium*, *Gnathoceros*, and *Trogoderma* introduced in the initial density of 30 each (15 males to 15 females). This mating was started primarily to test the technical feasibility of working with the three forms. However, since the results are of some interest in the light of the other experiments they are summarized briefly in Table 17 and Figure 14, where total population counts are listed for each species. The following points can be made:

1. *Trogoderma* becomes extinct in 120 days. In the face of such stringent competition there is essentially no reproduction detectable.
2. *Gnathoceros* and *Tribolium* develop somewhat erratically at first. The former has its usual large 30-day larval populations, and the latter is slow in getting started. During the 90–180-day interval *Tribolium* gets well established, and the *Gnathoceros* counts begin to decline. At the time of this writing the culture has run 450 days, and *Gnathoceros* is reduced to 8 larvae and 11 imagoes.

Competition and composition.—In the earlier treatment of the single-species controls the composition of the populations was discussed. Also, in dealing with certain of the

mixed populations, we pointed out here and there that one stage seemed to be affected by competition pressure while another stage seemed relatively immune. The latter were selected data; they covered events in the culture during a certain period of the life-cycle only, usually when the species was already well on its way to extinction.

TABLE 17

Tribolium-Gnathoceros-Trogoderma COMPETITION IN THE M-MATING

Age	*Tribolium*	*Gnathoceros*	*Trogoderma*
30	535	969	29
60	269	468	12
90	1,188	438	1
120	1,469	316	0
150	945	146	.....
180	1,799	151	.....
210	1,644	231	.....
240	1,093	249	.....
270	1,285	248	.....
300	1,822	106	.....
330	1,495	137	.....
360	2,858	90	.....
390	2,707	60	.....
420	3,205	31	.....
450	2,794	19	.....

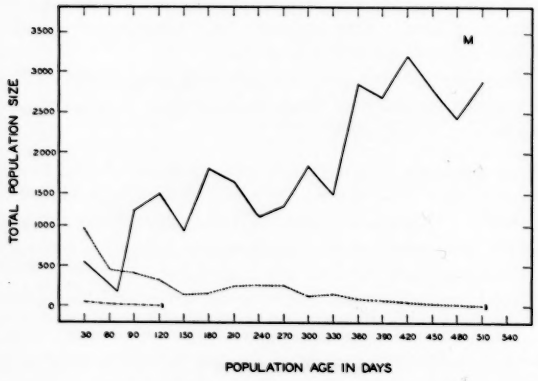


FIG. 14.—Population growth of *Tribolium-Gnathoceros-Trogoderma* culture (M-mating). Solid line, *Tribolium;* dotted line, *Gnathoceros*.

In this section we ask two questions: Is the composition during the total tenure of the experiment differentially altered depending on whether the species wins or loses to its associate, and are there any significant deviations in composition for any species when it is cultured with another form as compared with its control (isolated) growth? Both of these matters are considered in Table 18. This table tabulates experiment by experi-

TABLE 18

THE EFFECT OF COMPETITION ON COMPOSITION: SUMMARY OF ALL MATINGS FOR ALL THREE SPECIES

	Tribolium			*Gnathoceros*			*Trogoderma*		
	Larvae	Pupae	Imagoes	Larvae	Pupae	Imagoes	Larvae	Pupae	Imagoes
Percentage composition when species wins	68.1	5.1	26.2	88.2	1.8	10.0	91.5	1.2	7.3
	60.4	4.4	35.2	85.6	2.2	12.2	.....	.....	.....
	73.4	4.7	21.9	82.8	1.9	15.3	.....	.....	.....
	57.1	4.5	38.4	78.4	2.1	19.5	.....	.....	.....
	77.5	4.5	18.0	.....	.....	.....	.....	.....	.....
Mean (winners)	67.4	4.7	27.9	83.7	2.0	14.3	91.5	1.2	7.3
Percentage composition when species loses	75.9	6.7	17.4	91.3	1.0	7.7	83.8	0.6	15.6
	60.4	4.4	35.2	90.3	0.2	9.5	99.4	0.0	0.6
	.....	.....	.....	74.9	2.6	22.5	92.3	1.9	5.8
	.....	.....	.....	.....	.....	.....	96.3	0.3	3.4
	.....	.....	.....	.....	.....	.....	99.2	0.8	0.0
Mean (losers)	68.1	5.6	26.3	85.5	1.3	13.2	94.2	0.7	5.1
Mean (winners and losers)	67.6±2.01	4.9±0.18	27.5±2.04	84.5±1.45	1.7±0.18	13.8±1.30	93.7±1.71	0.8±0.2	5.5±1.43
Control (single species)	65.5±0.73	7.2±0.36	27.3±0.83	83.4±0.51	2.4±0.17	14.2±0.41	89.4±0.98	1.8±0.18	8.8±0.53

ment for each of the three species (1) the percentage of larvae, pupae, and imagoes when one species wins and the mean for that group; (2) the percentage when the same species loses and its mean; (3) the mean of winners *plus* losers; and (4) the control means taken from Tables 1, 4, and 7. It is recognized, of course, that the numbers are small—too small to add probable errors to certain means. This parameter is computed for the means of winners *plus* losers to allow comparison with control means. Despite the objection of small numbers the following points are worth mentioning:

1. There is no evidence in Table 18 that the percentage composition is affected by either winning or losing. There are some deviations, and, unfortunately, their significance cannot be appraised statistically. But at least we can say definitely that there are no marked alterations of composition that depend on the success or failure of a competition. In fact, the percentages are rather closer than one might expect a priori.
2. When the means for losers and winners are grouped (which procedure is permissible in the light of conclusion 1 just above) and compared with control means the differences in relation to their probable error result as shown in Table 19. These comparisons show that for *Tribolium* and *Gnathoceros* interspecific competition does not alter significantly the percentage of larvae or imagoes but seems to decrease the number of pupae, and for *Trogoderma* competition tends to increase the number of larvae and to decrease the number of pupae and imagoes. The latter findings check with earlier discussions of the *Trogoderma* matings.

TABLE 19

Stages	*Tribolium*	*Gnathoceros*	*Trogoderma*
Larvae	1.4	1.0	3.2
Pupae	8.5	4.4	5.5
Imagoes	0.1	0.4	3.2

DISCUSSION

Critique.—At this point it seems desirable to evaluate this investigation critically. Let us examine the technique employed and list its weaknesses and strengths as follows:

1. Debits
   a) Although no eggs (except some of *Trogoderma*) are lost, egg counts cannot be made because of the nature of the medium. It would be desirable to know how egg numbers vary from one count to another. This might give a closer approximation of productivity, as well as some information on cannibalism.
   b) More replications of certain matings would be desirable. Owing to the tedious character of the census method (one examination takes from 1 to 3 hours for two observers) it was impossible for us to maintain a more extensive experiment. Additional replications would smooth out the variation and strengthen the interpretations in certain spots. We do feel, however, that the investigation is extensive enough to warrant the drawing of accurate, descriptive conclusions.
   c) There is a disturbance element introduced into the experiment when the census is taken. Patently, this cannot be avoided, but we have tried to standardize handling so that this disturbance is essentially minimal and equivalent for each mating. The general consist-

ency of the results suggests that we were fairly successful in this effort. Our experience indicates that *Trogoderma* is more vulnerable to handling effects than either *Gnathoceros* or *Tribolium*.

2. Credits
   a) In addition to the close standardization mentioned above, the environment external to the culture bottle is controlled. Also, there are no critical differences in environment between one mating and another, since all bottles are kept in the same incubator.
   b) By renewing the medium at each 30-day count, conditioning is kept from being an important variable in this study. The competition measured is thus an expression of beetle behavior in an essentially optimum environment.
   c) The medium itself is closely standardized and reproducible.
   d) There is an accurate collection, counting, and recording of the beetles. Although the technique is laborious, it does not sacrifice reliability.
   e) In taking the census the population is not sampled but is counted in entirety. This rules out a source of variation and permits working with larger numbers.

In summary it seems judicious to say that, while our technique does have some obvious faults and most certainly can be improved, it does, nevertheless, permit the collection of an accurate body of quantitative knowledge about a biological topic little studied in any precise, experimental manner.

Problems.—This paper is the initial member in a series. The data discussed have frequently opened questions that require further analysis. The program will be well served if certain of these questions are delineated. Such delineation will aid in co-ordinating what has been said already and will also indicate general paths along which future analyses can travel. The following annotated outline focuses attention on some of these problems. This outline is illustrative rather than comprehensive.

I. Problems relating to the single-species populations
   A. *Tribolium*. The major problem raised is the oscillation cycle. Does it always occur? What are the causes? Earlier it was suggested that infection with a sporozoan, *Adelina*, may be responsible for the cycle. If this is true, an interesting opportunity exists for studying what Greenwood has called "experimental epidemiology" (see Greenwood, Hill, Topley, and Wilson, 1936). In our laboratory we have sterilized *Tribolium* of its parasites. This may furnish a tool for the analysis. In a sense such a study would be an example of interspecific competition—beetles with microörganisms.
   B. *Gnathoceros*
      1. Analysis of the "high-low" growth trend.
      2. Artificial increase of *Gnathoceros* density. It was noted that this species never builds up large populations like *Tribolium*. It would be interesting to increase the intraspecific population pressure by regularly adding new forms and observe the effect of this on the equilibrium and composition of the culture.
   C. *Trogoderma*
      1. The opportunity exists for analysis of population growth under conditions of "explosive" reproduction and short life-duration.
      2. The departure of the sex ratio in both *Gnathoceros* and *Trogoderma* from an expected 1 : 1 deserves analysis.

II. Problems relating to the mixed-species populations
   A. The effect of unequal sex ratios on population growth. This analysis would be concerned with male-female frequency as a factor in interspecific competition.
   B. More detailed analysis of "establishment" including its more precise definition, its experimental modification, and disturbance.

C. Selection experiments
   1. Selection induced by the physical environment. Varying such factors as temperature, humidity, or type of medium and assaying the effect of this on the mixed-species population.
   2. Selection primarily induced by biotic factors. Examples:
      a) The effect of cannibalism and other predation pressures on the population's growth. Cannibalism can be studied under normal (i.e., as it exists in the matings described in this paper) and artificial conditions. In the latter case the cannibalism would be manipulated in order to favor one species, etc.
      b) The effect of initial densities, different from those used here, on the competition. This problem introduces the question of wider density thresholds.
      c) Selection pressures created by artificially altering reproduction and mortality. For example, the addition of extra eggs, the removal of imagoes of certain age, etc.
      d) The possible effect of gases and secretions of beetle origin on both single and mixed-species populations.[6]

D. Migration effects. An attempt to approximate experimentally immigration and emigration by removing or adding beetles according to some logical system.

E. The effect of conditioning on population contraction. This applies to both single and mixed-species populations.

F. Analysis of possible co-operative effects either on the entire culture or one of its components.

There remains much to be said about the nature of competition pressures within populations, whether studied in the field, the laboratory, or from the viewpoint of rational mathematical analysis. The experiments reported here have described effects that are due primarily to certain aggregate behavior relationships among the beetles. Clements and Shelford (1939) have designated such relationships as "coactions"—the effect of organisms upon one another. Coactions invariably result whenever organisms come into close contact or aggregate. These coactions may take the following forms on the basis of their end effect on the population: first, they may affect adversely the physical environment (medium), in which case they are called "disoperation"; second, they may result in a more or less active demand by the population components on some substance or condition in the environment the supply of which is limited—"competition"; or, third, they may create a situation that has favorable aspects (e.g., increased survival value) for the coacting animals—"co-operation." In a recent paper Park (1941) reviewed these concepts critically and showed that many of the data of laboratory population studies can be interpreted in conjunction with this system.

The present study has described in quantitative terms certain descriptive events occurring in interspecific populations. The causes of these events are not definitely known. If they were, it would be instructive to discuss them in the light of a general ecological system such as that proposed by Clements and Shelford. Were this feasible, our data would be synthesized into a logical framework. Quantitative information would exist about the coactions that would permit their classification into descriptive, competitive, co-operative categories. It is not possible to do this now. We must await further experimental analysis. There is opportunity for much speculation at the moment, but the

[6] Mr. Louis Roth of New York University informs us (personal communication) that he has succeeded in obtaining crystals of a secretion of *Tribolium* that cause certain abnormalities during larval development. *Gnathoceros* and *Trogoderma* may also produce analogous materials, and it is conceivable that these substances may have an effect on population, as well as on individual, growth.

points still remain just that. Such factors as cannibalism and predation; aberration of metamorphosis, fecundity, and fertility; differential mortality; specific behavior patterns; competition for food and niches—all immediately suggest themselves as enticing partial explanations. At the moment we must content ourselves with the thought that this study or others like it may eventually reach such a stage. It is our opinion that ecology—and, by that token, biology—will be well served when and if such an end is attained.

As a final point it should be emphasized that, while we have discussed largely competitive coactions in this paper, excellent suggestions of co-operation also emerge from the data. For example, take the *Tribolium-Gnathoceros* case. There the ultimate success of the entire population appears to depend on the maintenance of a certain numerical density. If *Tribolium* gets too small as a culture, *Gnathoceros* is able to drive it out. Differently said, *Tribolium* is protected *as a population* by virtue of its own numbers. It is being recognized more and more that such co-operative effects grow out of aggregation and that they play major roles in species success and failure. This is true both for laboratory and for natural populations. Allee and his associates have implemented this idea frequently in the literature by presentation of evidence drawn from a catholic assembly of investigations.

SUMMARY

This investigation attempts to analyze experimentally the effect of interspecific competition on population growth. Three species of granary beetles—*Tribolium confusum*, *Gnathoceros cornutus*, and *Trogoderma versicolor*—are cultured in the same microcosm, and counts of total population size and larval, pupal, and imaginal composition are taken at regular intervals. The experiments consist of three parts: (1) control populations where each of the three species are cultured singly; (2) matings where all three species are cultured in all combinations of twos with both species seeded initially in equal density; and (3) matings where one species is introduced in numerical superiority over its competing mate. A detailed summary of major points appears in the body of the paper after each section. The following general conclusions describing the end result of the competition may be listed:

1. If *Tribolium* gets well established as a population, it drives out *Gnathoceros*.
2. If *Tribolium* populations are reduced to a low point by some type of epidemic infection (or, presumably, some other mechanism), *Gnathoceros* gets the upper hand and *Tribolium* becomes extinct.
3. *Tribolium* always drives out *Trogoderma*, irrespective of initial imaginal densities.
4. *Gnathoceros* usually drives out *Trogoderma*. This is true if the former is seeded initially in equal or higher densities. *Trogoderma* is the winner when started in a higher density than *Gnathoceros*.

ACKNOWLEDGMENTS.—The authors wish to acknowledge their real indebtedness to the late Professor Raymond Pearl, of the Johns Hopkins University, who was keenly interested in this project and who aided us in the design of the experiments. Our sincere thanks are also due to Professor W. C. Allee, of the University of Chicago, for similar counsel and for critically reading the manuscript, and to Professor Sewall G. Wright, of the University of Chicago, who read the manuscript and offered a number of helpful suggestions. Also we are indebted to Dr. Maud Norris, of the Imperial College of Science and Technology, London, and to Mr. G. H. Mansbridge, of the Stored Products Research Laboratory, Slough, England, for supplying us with cultures of *Trogoderma* and *Gnathoceros*.

LITERATURE CITED

ALLEE, W. C., and EVANS, GERTRUDE. 1937. Some effects of numbers present on the rate of cleavage and early development in *Arbacia*. Biol. Bull., **72**:217–232.

BODENHEIMER, F. S. 1938. Problems of animal ecology. London: Oxford Press. Pp. 183.

CHAPMAN, R. N. 1928. The quantitative analysis of environmental factors. Ecology, **9**:111–122.

———. 1933. The causes of fluctuations of populations of insects. Proc. Hawaii Ent. Soc., **8**:279–292.

CLEMENTS, F. E., and SHELFORD, V. E. 1939. Bio-ecology. New York: John Wiley & Sons. Pp. 425.

DICK, JOHN. 1937. Oviposition in certain Coleoptera. Ann. Applied Biol., **24**:762–796.

GAUSE, G. F. 1934. The struggle for existence. Baltimore: Williams & Wilkins. Pp. 163.

GAUSE, G. F.; SMARAGDOVA, N. P.; and WITT, A. A. 1936. Further studies on interaction between predators and prey. Jour. Anim. Ecol., **5**:1–18.

GREENWOOD, M.; HILL, A. B.; TOPLEY, W. W. C.; and WILSON, J. 1936. Experimental epidemiology. Medical Res. Council Rep. Ser., **209**:1–204.

JOHNSON, W. H. 1933. Effects of population density on the rate of reproduction in *Oxytricha*. Physiol. Zoöl., **6**:22–54.

LOTKA, A. J. 1934. Théorie analytique des associations biologiques. Actualités scientifiques et industrielles, **187**:1–45.

NICHOLSON, A. J. 1933. The balance of animal populations. Jour. Anim. Ecol., Suppl., **2**:132–178.

NORRIS, MAUD J. 1936. Experiments on some factors affecting fertility in *Trogoderma versicolor* Creutz. Jour. Anim. Ecol., **5**:19–22.

OMORI, N. 1939. Experimental studies on the cohabitation and crossing of two species of bed-bugs. Acta jap. med. trop., **1**:127–154.

PARK, THOMAS. 1934. Observations on the general biology of the flour beetle, *Tribolium confusum* Duval. Quart. Rev. Biol., **9**:36–54.

———. 1941. The laboratory population as a test of a comprehensive ecological system. *Ibid.*, Part I, Vol. **16**: No. 3; Part II, Vol. **16**: No. 4.

PARK, THOMAS, and WOOLLCOTT, NANCY. 1937. Studies in population physiology. VII. The relation of environmental conditioning to the decline of *Tribolium confusum* populations. Physiol. Zoöl., **10**: 197–211.

PEARL, RAYMOND. 1932. The influence of density of population upon egg production in *Drosophila melanogaster*. Jour. Exper. Zoöl., **63**:57–111.

REICH, KARL. 1936. Studies on the physiology of *Amoeba*. I. The relation between nutrient solution, zone of growth, and density of population. Physiol. Zoöl., **9**:254–263.

SMARAGDOVA, N. P. 1936. The interaction between phyrophagous and predatory mites in relation to their environment and concentration. Bull. Biol. and Med. Exper., **2**:99–100.

VLADIMEROVA, M. S., and SMIRNOV, E. S. 1938. The struggle for food in homogeneous and in mixed populations of *Musca domestica* and *Phormia groenlandica*. Meditsinskaia parazitologiia i parazitarnye bolezni, **7**:755–777.

VOLTERRA, VITO. 1928. Variations and fluctuations of the number of individuals in animal species living together. Jour. du conseil internat. pour l'exploration de la mer, Vol. **3**. Translated in CHAPMAN, Animal Ecology (1931), pp. 409–448.

GROWTH STUDIES OF COLPODA DUODENARIA. I. STERILIZATION OF THE CILIATES

C. V. TAYLOR AND W. J. VAN WAGTENDONK
School of Biological Sciences, Stanford University

RECENT investigations of Taylor, Brown, and Strickland (1936) and of Taylor and Strickland (1938, 1939) relating factors of the environment to the reactivities of *Colpoda duodenaria*, which involve its protoplasmic reorganization, have shown that it is desirable to standarize this experimental material. This follows from the general assumption that the developmental stage of the cell or oganism, as well as its genetic and environmental history, are indispensable components of the conditions necessary for reproducible results.

The first step in standardization is the cultivation of *C. duodenaria* in purely synthetic or at least well-defined and reproducible media. These organisms are grown in a balanced inorganic salt medium with no organic constituents other than the suspension of bacteria (a pure culture of a selected *Pseudomonas* sp. [?]) which is added as required for food. It is evident that this is by no means a defined medium. It was our aim to cultivate the *Colpoda* free from any other living organism.

Various methods have been described for the sterilization of Protozoa. Oehler (1919, 1920, 1924) was the first to report a bacteria-free culture of a ciliate. He obtained sterile individuals of *C. steinii* by isolating those that had migrated over the surface of an agar plate. He also obtained sterile cultures of *C. cucullus* by the same technique. Lwoff (1924) reported some data on cultivation of sterile *Glaucoma piriformis* but gave no details about the method used in the sterilization of this protozoan. Parpart (1928) sterilized *Paramecium caudatum* by means of repeated transfers of a single animal through sterile broth media. Peters (1929) claimed to have grown *C. colpoda* in bacteria-free cultures with inorganic nitrogen sources. However, his cultures actually contained bacteria (Gatenby and Cowdry, 1928). Butterfield (1929, 1933) obtained *Colpidium* (*G. piriformis*) in a sterile culture. Luck and Sheets (1931) made extensive efforts to sterilize cysts of *Euplotes taylori* by means of chemical reagents, but without success. The cysts were found to be less resistant to disinfectants than were the associated bacteria. Hetherington (1933) sterilized *C. campylum*, *G. scintillans*, and *Loxocephalus granulosus* by the washing method of Parpart. Elliott (1933) obtained *C. striatum* in a bacteria-free culture but did not give any details about the method used. In a recent paper Brown (1940) sterilized *Leucophrys patula* and *G. piriformis* by individual washing and migration through dilute agar (0.5 per cent or less).

By all these methods of sterilization it is possible to obtain only a few sterile ciliates at a time. It was desirable for our purposes to sterilize as many animals as possible in one treatment. This report will deal with attempts made to find such a method.

METHODS OF STERILIZATION

I. THE CENTRIFUGING METHOD

Kidder and Stuart (1939) described a simple method to get approximately 100 sterile *Colpoda* at one time. These were obtained by centrifuging and with much dilution of the *Colpoda* culture. This procedure was followed in essentials. Large numbers of *Colpoda* cysts[1] were excysted in 10 cc. sterile yeast extract (1.5 per cent Difco yeast). As soon as excystment started, the yeast extract was replaced by 10 cc. sterile balanced salt medium.[2] After complete excystment the solution, containing the free-swimming organisms, was transferred by means of sterile pipette to a sterile centrifuge tube (15 cc.), which was stoppered with a cloth-covered cotton plug. The ciliates were concentrated by slow centrifugation (1,200 r.p.m. during 5 minutes). Then 9 cc. of the solution were withdrawn and the tube was allowed to stand for about 5 minutes so that the ciliates might swim to the top of the remaining cubic centimeter of solution. With a sterile pipette 0.9 cc. of the ciliate suspension was withdrawn and placed in an empty sterile centrifuge tube. This suspension was diluted to 10 cc. with sterile distilled water, and the whole process was repeated until the ciliates had gone through twenty such transfers with the accompanying dilutions. This method entails a great loss of ciliates, and it failed to sterilize *C. duodenaria*. After innoculation of the ciliates thus washed twenty times into a yeast-glucose broth (1.5 per cent Difco yeast, 2 per cent glucose), prompt development of bacteria took place.

II. HEAT TREATMENT

Taylor and Strickland (1936) suggested that cysts of ciliates might be sterilized by exposure of dry cysts to high temperatures or to a high vacuum. This suggestion was followed, but without success. In brief, the procedure was as follows: a culture of *C. duodenaria* was centrifuged and washed three times under the conditions previously described. About 1 cc. of the concentrated suspension was placed in a Stender preparation dish. A sheet of cellophane was placed between the cover and the dish. Top and bottom were held together with a tight rubber band. The whole unit was autoclaved in a high Petri dish for 15 minutes at 130° C. After introduction of the ciliates the dish was inverted. The ciliates encysted on the cellophane. After complete encystment the sheet of cellophane was rinsed several times with sterile distilled water and dried under reduced pressure over P_2O_5. The sheet was then cut in small strips with sterile precautions and each strip placed in a sterile culture tube. These tubes were heated at different temperatures for lengths of time varying from 1 to 10 hours. After the heating, 2 cc.

[1] In all our experiments *C. duodenaria* were grown in sterile Petri dishes in order to avoid any air contamination.

[2] The balanced salt medium had the following composition:

Distilled water	100 cc.
NaCl	0.0104 gm.
$MgCl_2\ 6H_2O$	0.0085
$MgSO_4\ 7H_2O$	0.004
KCl	0.0023
$CaCl_2$ anh.	0.001

All chemicals were obtained from Merck quality reagent.

of the yeast-glucose medium were added to each tube. After complete excystment, 0.1 cc. of this excystment medium containing *Colpoda* was placed in a tube with yeast-glucose broth, and 0.1 cc. was plated on yeast-glucose agar.[3] As can be seen in Table 1 (showing only the results of the 10-hour heat treatment), the outcome of these experiments was discouraging. Not only were the results irregular (e.g., at 55° and at 65°), but heating of the dry cysts at 65° for 10 hours killed about 80 per cent of the cysts, while bacterial growth was visible after 3 days. Intermittent heat treatment did not solve the problem.

TABLE 1

Temperature (°C.)	Excystment*	Bacterial Growth†			
		1 Day	2 Days	3 Days	4 Days
40	+++++			250	
45	+++++		15		
50	+++++			130	
55	++++				25
60	+++			91	
65	+			115	

* Each + stands for 20 per cent excystment.

† Given in number of colonies on the agar plates. The tubes with yeast-glucose broth showed growth of bacteria on the same day.

Dry cysts were heated for 3 hours, excysted, and after centrifuging and subsequent washing (see Sec. I) were again made to encyst. These resulting cysts were killed by a second heating at 60° for 3 hours.

III. HIGH VACUUM TREATMENT

Heating at 60° and at a vacuum of 10^{-6} mm. Hg in a vacuum desiccator (after Abderhalden) for 4 hours and for 10 hours did not sterilize the cysts. In the first case bacterial growth was evident after 24 hours; in the latter after 48 hours. Excystment in the second case was diminished to 50 per cent.

IV. BACTERIOPHAGE

Broth media can be kept sterile by introducing with the bacteria a bacteriophage solution specific for that particular strain of bacteria. It seemed possible that a suitable bacteriophage would autolyze the bacteria and not affect the ciliates. It was found that the *Colpoda* were not affected by the presence of the phage but also that the phage failed to autolyze all the bacteria. Since bacteriophage affects bacteria only in the early growing stage, the technique finally used was as follows: *C. duodenaria* were cultured in the usual way. Instead of the *Pseudomonas* sp., *Listerella monocytogenes* (Schultz strain)

[3] Autoclaved 15 minutes at 120° C.

Difco yeast	7.5 gm.
Glucose	10
Agar (Difco)	10
Distilled water	500

(*L. monocytogenes*, K 12)[4] were given as food. After encystment the cysts were washed several times with sterile distilled water. Yeast-glucose broth was added and at the same time 0.2–0.5 cc. of the *L. monocytogenes* phage solution. Excystment was normal within 4 hours. The tubes stayed clear for 24 hours and in some cases even for 48 hours. The *Colpoda* multiplied during this period. But in all cases bacterial growth was visible within 48 hours. The control tubes (inoculated only with *L. monocytogenes* and the phage solution) remained clear and free from bacteria in all cases. There seem to be two possibilities: (1) *C. duodenaria* excrete a substance which inhibits the lysing action of the bacteriophage. (2) Some bacteria are not digested and become phage resistant by passing through the food vacuoles.

Since *L. monocytogenes* easily forms phage-resistant strains, the same experiments were repeated with two strains of *Staphylococcus aureus: Staph. aureus* AD 8 and *Staph. aureus* AD 11 together with the bacteriophage specific for these strains, Phage K 115.[5] The results,

TABLE 2

Time in Minutes	Excystment	Bacteria
2	Normal	Growth within 48 hours
4	Normal	Growth within 48 hours
8	Normal	Growth within 48 hours
10	Normal	Growth within 48 hours
14	Delayed	Growth within 48 hours
18	Delayed, 50 per cent excystment	Growth within 48 hours
20	No excystment	Sterile

however, were the same as those obtained with *L. monocytogenes*. An intermittent addition of phage solution was also unsuccessful. Cysts were treated with yeast-glucose broth with additional phage solution. After complete excystment the solution was centrifuged and the animals washed three times (see Sec. I). The *Colpoda* were then placed in a fresh yeast-glucose broth with 0.5 cc. phage solution. After 3 hours the whole procedure was repeated, and 3 hours later it was repeated again. In this last tube bacterial growth again took place within 48 hours.

V. ULTRAVIOLET IRRADIATION

Giese and Leighton (1935) studied the influence of ultraviolet radiation upon *Paramecia*. *Paramecia* did not die when irradiated with high dosages of intense light of wave length 3130 Å. By using shorter wave lengths, vesiculation occurred when between 10^{12} and 10^{13} quanta had been adsorbed by a *Paramecium*.

For the following tests a mercury lamp, type H_4 (G.E.), was used. The *C. duodenaria* were cultivated in quartz dishes. After encystment the cysts were washed several times with sterile distilled water and irradiated in the wet state. An irradiation time of 20 minutes (Table 2) killed both *Colpoda* and bacteria, while an irradiation of 18 minutes delayed and decreased the excystment and apparently did not injure the bacteria.

[4] We obtained *L. monocytogenes* K 12 and the phage K 12 from Professor C. E. Clifton, for which acknowledgment is hereby gratefully made.

[5] These were also obtained from Professor C. E. Clifton.

VI. PARPART'S METHOD (MODIFIED)

Raper (1937) has published a method of freeing fungi from bacterial contamination. He used a 2 per cent agar medium and let the fungi grow through the agar.

Combining this method with Parpart's washing method produced excellent results. Instead of the sterile water drops used by Parpart, sterile drops of a 0.5 per cent agar solution were used. After migrating through 3 drops, each having a diameter of approximately 3 mm., single individuals were transferred to a yeast-glucose broth. The tubes did not show any bacterial growth even after 7 weeks' incubation. During this period 0.1 cc. of the broth was plated out several times and 0.1 cc. transferred to fresh broth, but no bacterial growth could be observed. The *Colpoda*, however, did not multiply. It is therefore possible to sterilize *C. duodenaria* by this technique, but only one ciliate at a time could thus be obtained. Therefore, an attempt was made to utilize this principle of migration through agar in obtaining large numbers of sterile *Colpoda*. Since *C. duodenaria* always tend to swim to the surface of the medium, these ciliates were introduced at the bottom of an agar column in a glass tube. They migrated to the surface of the agar. During this passage the bacteria on the surface of the *Colpoda* were scraped off. Bacteria which were in the food vacuoles were supposedly digested by the *Colpoda* during the migration through the agar solution.

The technique which finally proved to be the most satisfactory and which made possible the sterilization of as many as 50 animals in one treatment was as follows: a glass tube of 15-cm. length and 7-mm. diameter was closed at one end with a serum cap and filled with a 0.35 per cent agar solution. The agar column was 75 mm. long. The tube was closed with a cotton plug and sterilized in the autoclave. A concentrated culture of *C. duodenaria* was centrifuged and washed three times as described before. The concentrate was then centrifuged through a sterile agar solution (0.35 per cent) in a 15-cc. centrifuge tube. This was done at such a speed that most of the *Colpoda* were thrown down in about 2 hours. The upper part of the agar was withdrawn by means of a sterile pipette. The remaining 0.5 cc. was injected with a sterile syringe through the serum cap. The *Colpoda* were then at the bottom of the agar column. The tube was left in a vertical position for 6–7 hours. At the end of that time enough *Colpoda* had migrated to the surface of the agar column to be removed. This was done by means of a sterile micropipette. The *Colpoda* were transferred to tubes containing yeast-glucose broth and were observed for 7 weeks. Frequently during that time 0.1 cc. was plated onto yeast-glucose agar and also 0.1 cc. innoculated into fresh yeast-glucose broth to test for bacteria. No bacterial growth was observed during the 7 weeks, and no bacteria could be detected on microscopical examination of the broth cultures. Therefore it may be assumed that these *Colpoda* were sterile.

SUMMARY

Several methods, described in the literature for sterilizing ciliates, proved unsatisfactory for the sterilizing of *C. duodenaria*. A new method is described which involves migration of *Colpoda* through a sterile viscous agar medium. The *C. duodenaria* so obtained were rigorously tested for sterility during 7 weeks after the migration through the agar and proved to be sterile.

LITERATURE CITED

BROWN, M. G. 1940. Growth of protozoan cultures. II. *Leucophrys patula* and *Glaucoma piriformis* in a bacteria-free medium. Physiol. Zoöl., **13**:277.

BUTTERFIELD, C. T. 1929. III. A note on the relation between food concentration in liquid media and bacterial growth. U.S. Public Health Repts., **44**:2865.

———. 1933. A note on the food habits of *Colpidium*. *Ibid.*, **48**:814.

ELLIOTT, A. M. 1933. Isolation of *Colpidium striatum* Stokes in bacteria-free media. Biol. Bull., **65**:45.
GATENBY, J. B., and COWDRY, E. V. 1928. Bolles Lee's Microtomist's Vade mecum. Philadelphia: Blakiston.
GIESE, A. C., and LEIGHTON, P. A. 1935. Quantitative studies on the photolethal effect of quartz ultraviolet radiation upon *Paramecium*. Jour. Gen. Physiol., **18**:557.
HETHERINGTON, A. 1933. The culture of some holotrichous ciliates. Arch. f. Protistenk., **80**:255.
KIDDER, G. W., and STUART, C. A. 1939. Growth studies on ciliates. I. The role of bacteria in the growth and reproduction of *Colpoda*. Physiol. Zoöl., **12**:329.
LUCK, J. M., and SHEETS, G. 1931. The sterilization of Protozoa. Arch. f. Protistenk., **90**:396.
LWOFF, A. 1924. Le Pouvoir de synthèse d'un protiste hétérotrophe: *Glaucoma piriformis*. Compt. rend. Soc. biol., **91**:344.
OEHLER, R. 1919. Flagellaten und Ciliatenzucht auf reinem Boden. Arch. f. Protistenk., **40**:16.
———. 1920. Gereinigte Ciliatenzucht. *Ibid.*, **41**:16.
———. 1924. Gereinigte Zucht von freilebenden Amöben, Flagellaten und Ciliaten. *Ibid.*, **49**:287.
PARPART, A. K. 1928. The bacteriological sterilization of *Paramecium*. Biol. Bull., **55**:113.
PETERS, R. A. 1929. Observation upon the oxygen consumption of *C. colpoda*. Jour. Physiol., **68**:1.
RAPER, J. R. 1937. A method of freeing fungi from bacterial contamination. Science, **85**:342.
TAYLOR, C. V., BROWN, M. G., and STRICKLAND, A. G. R. 1936. Effects of a given X-ray dose on cysts of *Colpoda steinii* at successive stages of their induced excystment. Jour. Cell. and Comp. Physiol., **9**:105.
TAYLOR, C. V., and STRICKLAND, A. G. R. 1936. Effects of high vacua and extreme temperatures on cysts of *Colpoda cucullus*. Physiol. Zoöl., **9**:15.
———. 1938. Reactions of *Colpoda duodenaria* to environmental factors. I. Some factors influencing growth and encystment. Arch. f. Protistenk., **90**:396.
———. 1939. Reactions of *Colpoda duodenaria* to environmental factors. II. Factors influencing the formation of resting cysts. Physiol. Zoöl., **12**:219.

EXPERIMENTAL STUDIES ON A JAPANESE PLANARIAN
II. AXIAL DIFFERENTIAL IN RATE OF EYE FORMATION IN RECONSTITUTION

(Three figures)

YISAMU WATANABE
Nakajima Park, Sapporo, Japan

IN AN earlier paper by Child (1932) it was shown that a Japanese triclad known as *Planaria gonocephala*[1] resembles two American species, *Dugesia* (=*Euplanaria*) *dorotocephala* and *D. tigrina* (=*E. maculata*)[2] in possessing a definite fission zone and a physiologically well-defined posterior zooid region and in showing differential susceptibility along the longitudinal axis of the body to toxic concentrations of various agents used. In these three species the susceptibility is highest at the anterior end and decreases posteriorly to the level of fission but increases more or less again in the posterior zooid region. In the two American species the data on head frequency—i.e., capacity for and degree of head reconstitution—agree with those on susceptibility to lethal agents not only in indicating the existence of an axial physiological differential but also as regards its general form (Child and Watanabe, 1935; Watanabe, 1935*b*; and citations). In the present species, as has been pointed out (Watanabe, 1937, p. 294, n.), head frequency is extremely high and even 1/16 pieces from any level all develop normal heads. Consequently, no axial differential can be detected. However, it would be premature to deny the existence of an axial differential in the activity of reconstitution in this species on the basis of this evidence, because, besides head frequency, there is another important factor in head development, namely, rate of differentiation which may be regarded as representing an intensity factor in certain reconstitutional processes.

Data on the axial differential in rate of head development as indicated by time of appearance of eyespots have been recently given for several species of single-zooid planarians.[3] But, as to the planarians which undergo fission and have a distinct posterior zooid region, the data at hand are for only the one species, *D. dorotocephala* (Watanabe, 1935*a*). In pieces of given length of this species eyespots appear the later, the more posterior the level of head formation within the anterior zooid. Also heads with abnormal eyes (connected eyes and single median eyes), which appear in shorter pieces from the more posterior pieces of the anterior zooid, develop less rapidly than those with normal symmetrical eyes. Evidently, rate of eye formation and head frequency are associated in *D.*

[1] Although this species is called *Planaria gonocephala*, it differs widely as regards physiological characteristics from the European *P. gonocephala* (Abeloos, 1930) and is probably a different species.

[2] According to Hyman (1931), American species of the genus earlier called *Planaria* must be called *Euplanaria*. More recently (Hyman, 1939) she has stated that the genus name *Dugesia* has priority over *Euplanaria*. Since the genus is closely related to the European *Planaria* and members of the group have long been known as planarians, *Euplanaria* is a far more satisfactory designation. The result of strict application of the rule of priority seems particularly unfortunate in this case.

[3] *Planaria gonocephala* (Abeloos, 1927, 1930), *Planaria lugubris* (Sivičkis, 1933, 1934), *Dendrocoelum lacteum* (Sivičkis, 1931*b*), *Polycelis nigra* (Sivičkis, 1933), *Phagocata gracilis* (Buchanan, 1933).

dorotocephala. For this reason it was thought desirable to work with other planarians in which the head-frequency factor can be eliminated even in short pieces. In this respect the Japanese species provides very favorable material. The present investigation is, then, concerned with the question of the relation of rate of head development to level of body and length of piece as indicated by time of appearance of eyespots and quite independently of head frequency. To Dr. C. M. Child, of Stanford University, thanks are due for his constant encouragement and helpful suggestions.

MATERIAL AND METHOD

The worms were collected from spring-fed streams in and about the city of Sapporo. For the experiment animals which had not recently undergone fission were selected and

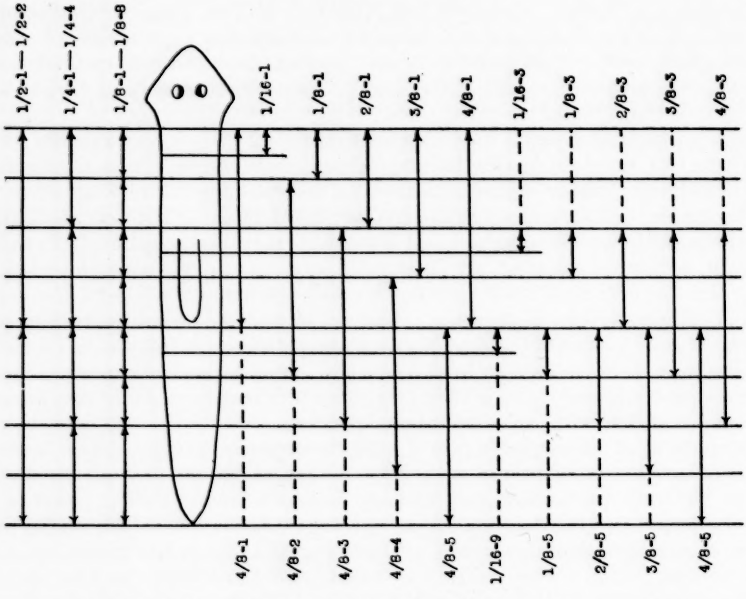


FIG. 1.—Diagram showing pieces, lengths, and levels of body used

were kept without food 5 days before experiment. In most cases lots of 20 pieces each were used, and the experiment was repeated to a total of 200 pieces. Pieces were cut transversely with a small scalpel, and each 20 pieces from the same body-level were placed together in 200 cc. of fresh well water—i.e., 10 cc. of water for each piece—and kept in a dark cabinet without change of water. The temperature in the cabinet was controlled by thermostat. Temperature variation was not over 3.0° C., in each case.

Determination of presence or absence of developed eyespots was made at 8- or 12-hour intervals with compound microscope, magnification of fifty, and careful focusing below the epithelium; eyespots were distinguishable from the body pigment by their deeper

location. From the numbers of pieces with eyespots thus recorded the arithmetical means of time from section to appearance and average standard deviations within lots were calculated. The statistical probability (P) that as great a difference might be given by random sampling was also calculated with use of "Students' " method and a table of t (Fisher and Yates, 1938).

The pieces of equal length from different levels of the same bodies are indicated numerically according to the fraction of the postcephalic length which the pieces represent—1/2, 1/4, 1/8—and the body-level by a numeral following the fraction, as 1/2-1 to 1/2-2, 1/4-1 to 1/4-4, and 1/8-1 to 1/8-8, the level 1 being the anterior level just behind the head. Pieces of different lengths with anterior ends at the same level are designated as follows: 1/16-1, 1/8-1, 2/8-1 to 4/8-1; 1/16-5, 1/8-3, 2/8-3 to 4/8-3; 1/16-9, 1/8-5, 2/8-5 to 4/8-5, the numeral following the fraction indicating the level of the anterior cut end of the piece. Pieces of equal length from different levels but so long that they are of necessity from different animals—e.g., 4/8 pieces—are designated 4/8-1, 4/8-2 to 4/8-5. The various pieces, lengths, and levels used are indicated in Figure 1.

EXPERIMENTAL RESULTS

Times of appearance of eyespots in pieces of equal length from different levels of the same animals.—In these experiments the mean times of appearance of eyespots of 1/2, 1/4, and 1/8 pieces were determined in animals of 9–10 mm. and 14–15 mm. in length. The material of the two different lengths was collected at different times of year.

As the data given in Table 1 show, the mean time of appearance of eyespots is least at the most anterior level and increases to the middle region but decreases again at more posterior levels. In other words, the rate of eye formation is highest at the most anterior level and decreases toward the posterior end of the anterior zooid but again increases in the posterior zooid region (cf. the last column of Table 1). The values of statistical probability, P, for difference of average mean times between successive levels indicate high significance in the anterior levels and less significance in the middle levels. Such anteroposterior variation in P is undoubtedly dependent on the greater variation in mean times of the more posterior pieces from the anterior zooid. In each series, with some exceptions, average standard deviation of mean times increases also from anterior to posterior levels of the anterior zooid and decreases again in the posterior zooid region.

Rate of eye formation of 1/8 pieces from an abnormal stock.—Even though the reconstitution of pieces takes place at the same temperature, the rate of eye formation shows some variations with different stocks of worms collected at different times of year, from different localities, and kept for different lengths of time in the laboratory. Nevertheless, the axial relation of rate to level of body is usually similar to that described above. However, the rates in certain worms collected from one locality in midsummer of 1939 were almost identical at all levels of body. The mean times and rates of 1/8 pieces from these worms of 14–15 mm. in length are shown in Table 2.

The animals of this stock were apparently, for some unknown reason, not in normal condition. One month after the collection many individuals began to disintegrate, and during the following two weeks all died, though stocks usually live indefinitely under laboratory conditions. These anomalies apparently represented a temporary condition, because worms from other collections from the same spring-fed stream did not show them.

Effect of length of piece on rate of eye formation.—The data in Table 1 suggests that the rate at a given level decreases with decrease in piece length. In order to obtain more ac-

TABLE 1

MEAN TIMES IN HOURS TO APPEARANCE OF EYES IN 1/2, 1/4, AND 1/8 PIECES

Data are averages of ten lots, each of pieces from 20 worms, a total of 200 worms. Examinations were made at 8-hour intervals.

Body Length (In Mm.)	Temperature Range (° C.)	Pieces	Average Mean Times	Average Standard Deviations	*P*	Relative Rate in Percentages
9–10	27–30	1/2-1	48.9	4.97	<0.001	100.0
		1/2-2	54.2	7.09		90.3
		1/4-1	49.6	5.34	<0.001	100.0
		1/4-2	54.2	6.70	0.002	91.3
		1/4-3	55.7	7.01	0.018	89.4
		1/4-4	54.9	6.89		90.7
		1/8-1	48.0	4.37	<0.001	100.0
		1/8-2	50.1	5.48	<0.001	95.8
		1/8-3	53.6	6.94	0.006	89.6
		1/8-4	55.5	6.92	0.168	86.5
		1/8-5	56.2	7.15	0.011	85.4
		1/8-6	58.1	6.62	0.803	88.8
		1/8-7	53.9	7.10	0.061	89.1
		1/8-8	52.8	5.78		90.9
14–15	27–30	1/2-1	49.1	5.04	<0.001	100.0
		1/2-2	55.7	6.58		88.4
		1/4-1	50.6	6.70	<0.001	100.0
		1/4-2	56.0	8.62	<0.001	90.3
		1/4-3	60.1	8.85	<0.001	84.3
		1/4-4	58.2	8.93		87.0
	25–28	1/8-1	50.6	6.29	0.005	100.0
		1/8-2	53.0	7.00	<0.001	95.5
		1/8-3	56.9	7.30	0.006	89.3
		1/8-4	59.4	7.42	0.465	85.3
		1/8-5	60.2	7.77	0.003	84.1
		1/8-6	57.0	7.59	0.064	88.7
		1/8-7	56.0	8.25	0.055	90.3
		1/8-8	55.0	7.95		92.0

curate experimental evidence concerning the relation between rate of eye formation and length of piece, the mean times of pieces of five lengths—1/16, 1/8, 2/8, 3/8, and 4/8—with the anterior ends at the same levels (Fig. 1) were determined, and from these means the rates of the pieces relative to 1/8 pieces from the same individuals were calculated, except at the level 1, where the relative rates were determined as percentages of the rates of 1/8 pieces from all other individuals of the same lots. Pieces with anterior ends at the same levels came from the same stock, but those for different levels from different stocks. Consequently, only the data for the pieces from the same levels are directly comparable.

The data in Table 3 show, first, that, at the anterior level immediately behind the

TABLE 2

MEAN TIMES IN HOURS TO APPEARANCE OF EYES IN 1/8 PIECES FROM AN ABERRANT STOCK

Data are averages of ten lots, each consisting of pieces from 20 worms of 14–15 mm. in length: 200 worms; temperature range: 27°–30° C. Examinations at 8-hour intervals.

Pieces	Average Mean Times	Average Standard Deviations	*P*	Relative Rate in Percentages
1/8-1	45.9	2.49		100.0
			0.017	
1/8-2	46.7	3.31		98.3
			0.001	
1/8-3	48.5	4.42		94.6
			0.190	
1/8-4	47.9	4.38		95.8
			0.336	
1/8-5	47.7	4.65		96.2
			0.144	
1/8-6	47.0	3.52		97.7
			0.097	
1/8-7	46.0	3.24		99.8
			<0.001	
1/8-8	47.3	3.40		97.0

head, rates of eye formation are nearly equal for pieces of different lengths and, second, that at the more posterior levels there is in general a continuous increase with increase in length of piece from 1/16 to 3/8 and no appreciable increase in rate from 3/8 to 4/8. The values of *P* indicate that, at the level just behind the head, differences for the pieces of increasing length are not significant, but at the posterior levels of the anterior zooid those for 1/16-5 and 1/8-3, 1/8-3 and 2/8-3, 2/8-3 and 3/8-3, 1/16-9 and 1/8-5, 1/8-5 and 2/8-5 are all significantly different. As shown in the last column of the table, average standard deviation, with a few exceptions, decreases as the length of piece increases at the posterior levels, while it does not appreciably change at the most anterior level.

Effect of posterior half-section on rate of eye formation.—These experiments are supplementary to the preceding. As shown above, in posterior pieces from the anterior zooid the rate of eye formation decreases with decrease in length of piece. If this is due to the effect of length of piece or to a stimulation from the posterior section, it should be possible to retard the rate of eye formation on one side of the piece by making a posterior

half-section a short distance posterior to the anterior section. The following experiments were undertaken in order to test this possibility.

In 1/2 pieces from animals 18–20 mm. in length a transverse section was made from one side of the body to the median line 1.5 mm. posterior to the anterior section (Fig. 2), or a strip of tissue half the width of the body was removed posterior to this half-section

TABLE 3

RATE OF EYE FORMATION AT THREE DIFFERENT LEVELS OF BODY IN RELATION TO LENGTH OF PIECE

Data are averages of ten lots, each of 20 pieces, for each length from 12–13 mm. worms, i.e., 1,000 worms for each level. Examinations at 8-hour intervals.

Temperature (° C.)	Pieces	Relative Rate in Percentages	*P*	Average Standard Deviations (In Hours)
24–27	1/16-1	100.1	>0.900	11.5
	1/8-1	100.0	0.404	11.7
	2/8-1	99.4	>0.900	11.6
	3/8-1	99.5	0.369	11.8
	4/8-1	99.9		11.6
19–22	1/16-5	81.6	0.004	14.2
	1/8-3	86.5	0.038	14.2
	2/8-3	89.6	0.042	13.3
	3/8-3	92.8	0.500	13.6
	4/8-4	93.0		13.0
	1/16-9	77.8	<0.001	12.1
	1/8-5	84.5	0.021	11.9
	2/8-5	87.6	0.161	11.9
	3/8-5	90.0	0.271	10.6
	4/8-5	90.8		10.1

(Fig. 3). Pieces used for a single half-section and those for removal of tissue came from different stocks. Results are given in Table 4.

In anterior 1/2 pieces, with or without removal of tissue, average mean times of appearance of eyespots are slightly longer on the side of half-section than on the intact half, but the differences in time are less than 1 hour and entirely without significance as indicated by high values of statistical probability, *P*. In posterior 1/2 pieces average mean times of the side of half-section are 5–7 hours longer than those of the other side.

Though such differences seem to be rather small as compared with the mean times, the values of P for these differences indicate that they are unquestionably highly significant. That is to say, at the anterior level immediately posterior to the head the half-section does not affect the rate of eye formation, but at the posterior level a retardation of eye formation is clearly seen on the side of the half-section.

In this series the number of pieces which developed one eyespot on one side of the piece but none on the other was recorded at the time of examination of pieces. Percentages of such pieces are appended in the last two columns of Table 4: the column headed "Asymmetry" indicates cases in which the eyespot developed later on the side of half-section than on the side without half-section, and the column "Reversed Asymmetry," cases in which the eyespot developed earlier on the side of half-section than on the other. These percentages show also a distinct retardation of eye formation on the side of half-section at the posterior level.

Effect of posterior half-section upon the symmetry of head forms.—Retardation of development on the side of half-section makes the new head temporarily asymmetrical not only in time of appearance of eyespots but also in size of developed eyespots, amount of new tissue, and shape of lateral lobes (cf. Child and Watanabe, 1935, Figs. 12–29). For more precise determination of the percentages of symmetrical, asymmetrical, and reversed asymmetrical heads, another set of 1/2 pieces was prepared with the same procedures as in the preceding series and was examined at much shorter time intervals (less than 6 hours). Table 5 gives the results.

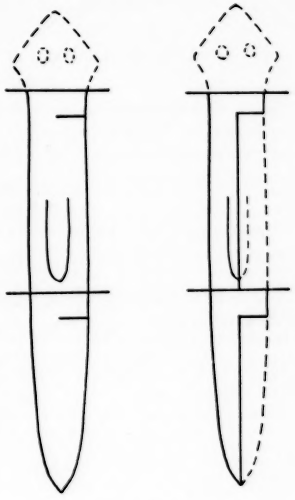


FIG. 2.—Posterior half-sections at anterior and posterior levels, without removal of tissue.

FIG. 3.—Posterior half-sections at anterior and posterior levels, with removal of tissue.

Table 5 shows that both with and without removal of tissue 64 and 65 per cent of heads at anterior level are symmetrical in all respects. At the posterior level, however, development is retarded on the side of half-section in 64 and 66 per cent. Reversed asymmetry occurs in 16 and 20 per cent at the anterior level, and in 7 and 10 per cent at the posterior level. Statistical probability, P, for difference in percentages between asymmetry and reversed asymmetry at the anterior level is 0.212 without removal of tissue and 0.209 with removal of tissue. These differences have little significance. Consequently, occurrence of asymmetry and reversed asymmetry at the anterior level is probably due to incidental factors having no relation to the half-section. At the posterior level, however, the values of P for the difference between asymmetry and reversed asymmetry are less than 0.001, that is, highly significant. All these data make it clear that at the posterior level a half-section and removal of a strip half the width of the body posterior to the half-section retard the development on the side of the injury but are not effective at the anterior level just behind the head.

Rates of eye formation in 4/8 pieces from different levels.—The preceding series have shown that the rate of eye formation varies with length of piece as well as with level of body. In the present series the rates of 4/8 pieces from five different levels of body were determined in order to eliminate length of piece as a factor in rate of eye formation and to show whether an axial differential independent of relation to piece length is present. The five levels of 4/8 pieces used differ by 1/8 of the postcephalic length (Fig. 1). The 4/8 pieces were necessarily taken from different individuals, but the mean times were

TABLE 4

RATE OF EYE FORMATION IN 1/2 PIECES AFTER POSTERIOR HALF-SECTION WITH AND WITHOUT REMOVAL OF TISSUE

Data are averages of ten lots, each of pieces from worms 18–20 mm. in length. Examinations at 12-hour intervals. Temperature: 9°–10° C.

Operation	Pieces	Average Mean Times in Hours		Difference		Asymmetry in Per-centages	Reversed Asymmetry in Per-centages
		Half-cut side	Intact side	In Hours	*P*		
Without removal of tissue	1/2-1	123.0	123.6	0.6	0.726	9	84
	1/2-2	134.6	139.6	5.0	0.020	35	
With removal of tissue	1/2-1	126.1	126.9	0.8	0.9	15	7
	1/2-2	144.6	151.6	7.0	0.008	55	4

TABLE 5

HEAD FORMS DEVELOPED (IN PERCENTAGES) AFTER POSTERIOR HALF-SECTION

Data are averages of five lots, each consisting of 20 pieces, from worms 18–20 mm. in length. Temperature: 12°–14° C.

Operation	Pieces	Symmetry	Asymmetry	Reversed Asymmetry
Without removal of tissue	1/2-1	65	15	20
	1/2-2	26	64	10
With removal of tissue	1/2-1	64	20	16
	1/2-2	27	66	7

also determined for 1/8-1 pieces of the same individuals, except in the case of 4/8-1 pieces where this is impossible, and the rates were calculated relative to these 1/8-1 pieces as a standard. For 4/8-1 pieces the rates were calculated from the mean times of 4/8-1 pieces and averages of four mean times of 1/8-1 pieces from individuals used for 4/8-2 to 4/8-5 pieces of the same lots. With these long pieces less difference in rate between anterior 4/8 and posterior 4/8 pieces was expected than with shorter pieces. For this experiment a stock was selected by preliminary examination showing a relatively

great difference in rate between pieces 4/8-1 and 4/8-5.[4] In Table 6 average rates, values of P, and average standard deviations obtained are given.

From the data of Table 6 it is clearly seen that average rates show a continuous decrease from the anterior to the posterior level. The values of P indicate, however, that the differences for the first and second and for the second and third 4/8 pieces are clearly significant, while those for the third and fourth and for the fourth and fifth 4/8 pieces are less significant. Average standard deviation of mean times increases from anterior to posterior levels of anterior zooid, with an exception in 4/8-5 pieces.

TABLE 6

RATE OF EYE FORMATION IN 4/8 PIECES FROM FIVE DIFFERENT BODY-LEVELS

Data are averages of ten lots, each consisting of 20 pieces for each level from worms 13–14 mm. in length: total, 1,000 worms. Temperature: 16°–19° C. Examinations at 12-hour intervals.

Pieces	Relative Rate in Percentages	P	Average Standard Deviations (In Hours)
4/8-1.....	99.9		13.2
		<0.001	
4/8-2.....	94.8		17.1
		0.021	
4/8-3.....	89.9		19.3
		0.080	
4/8-4.....	86.9		21.2
		0.085	
4/8-5.....	84.5		20.0

DISCUSSION

In considering the data presented above it is seen that the mean times of appearance of eyespots and the relative rates as percentages of a mean time taken as representing a standard rate differ in definite and orderly ways with level of body and length of piece. As noted in an earlier paper (Watanabe, 1935*a*), the relative rates do not involve any assumptions as to constancy of rate from section to appearance of eyespots but merely show certain relations of mean times and indicate only difference in rate of a series of changes which occur between section and appearance.

The data of Table 1 indicate that the rate of eye formation decreases in general from anterior to posterior levels of the anterior zooid and increases at more posterior levels. But the posterior rise is not always statistically significant. This axial relation of rate is similar to that of head frequency (Child and Watanabe, 1935) and of the eye formation (Watanabe, 1935*a*) in *D. dorotocephala*. It appears, then, that the rate of eye formation as an indicator of rate of head development gives evidence for the existence of a physiological differential along the longitudinal axis of body at least in the anterior zooid region.

[4] However, such difference in rate between 4/8-1 and 4/8-5 pieces as mentioned below is not unusual but is within the range of normal variations. In this species a difference as great as this has been frequently found in later experiments (unpublished data).

According to Sivičkis' classification of form of axial gradients in triclads (Sivičkis, 1931*a*), the present species seems to belong to his *P. velata* group rather than to his *P. dorotocephala* group, since the pieces taken from any level of this species produce 100 per cent of perfectly reconstituted individuals. But, as described above, the rate of eye formation of this species shows a distinct U-shaped double gradient with the lowest rate at or near the zone of fission. In Sivičkis' classification this U-shaped gradient is that which has been taken as a characteristic feature of the *dorotocephala* group. However, the only criterion used for his classification was the capacity for development of eyespots, no other characters in reconstitution being taken into account, though, as he insisted, the appearance of eyespots may be a convenient index, but not necessarily the best, for determination of the form of physiological axial gradients. In fact a gradient of capacity for head development is merely one of the various expressions of the axial differential in physiological organization of the planarian body. In addition to the data presented in this paper, the evidence from the growth rate of anterior and posterior new tissues and from differential susceptibility to lethal solutions as indicated by the disintegration processes in whole animals (Child, 1932) shows that the present material belongs to the *dorotocephala* group, although its head frequencies are extremely high and show no distinct axial differential as do those of other members of the group (cf. Child and Watanabe, 1935; Watanabe, 1935*b;* Sivičkis, 1923).

In normal worms the variations in steepness of the gradient of rate are usually limited within a certain range under average normal conditions of laboratory environment. However, as shown in Table 2, in a certain stock the rate was nearly equal at all levels of the body. Similar decrease in gradient has also been found in head frequency in *D. dorotocephala* in certain stocks the head frequency of which was extraordinarily high at all levels with very slight gradient (Watanabe, 1941). The worms of this stock were evidently not in normal condition. They did not live long under laboratory conditions, while worms of other stocks collected from the same locality lived indefinitely there and showed similar gradients of rate of eye formation to those in worms from other localities. Such anomalies probably result from certain conditions in the environment in nature prior to experimentation.

Abeloos and Buchanan are of opinion that the rate of head development has no relation to length of piece in *Planaria gonocephala* (Abeloos, 1930) and *Phagocata gracilis* (Buchanan, 1933). But the data presented by these authors do not appear to exclude the possibility of a relation between length of piece and rate of head development. As has been shown in Table 3, in the present species as in *D. dorotocephala* the rate is little or not at all affected by length of piece at the anterior level, but at more posterior levels of the anterior zooid it increases with increase in length of piece and attains a maximal value when the length of piece extends beyond 3/8 of the postcephalic length. These data indicate that, at the posterior levels of the anterior zooid, the differentiation processes in the formative tissue at or near the anterior cut surface are more or less retarded by a factor originating from the posterior section of the piece. The inhibitory action on head development apparently undergoes a decrement in effectiveness with increasing distance from the level of origin. This hypothesis is supported also by the results from posterior half-section (Tables 4 and 5). At the posterior level of the anterior zooid half-section made at a short distance posterior to the anterior cut retards head differentiation and growth of new anterior tissue on the side of the half-section, resulting in a temporary asymmetry in head form of the regenerates.

According to this view, the maximal rate of such long pieces as 3/8 and 4/8 of the post-

cephalic length indicates the rate of differentiation of the head-forming tissue itself at the level of the anterior cut without any effect of the inhibiting factor. Consequently, an axial differential of rate of eye formation in 4/8 pieces from five different levels (Table 6) indicates the existence of a gradient of head-differentiating activity along the main axis. This finding seems to be rather significant for the analysis of axial gradients of reconstitution in planarians.

It has been suggested in work with *D. dorotocephala* and *D. tigrina* that the head-frequency gradient is a result of axial differentials of the relative intensities of two antagonistic factors which are concerned in reconstitution of the planarian head: the one, a positive determining factor being the condition and activity of the cells which give rise to the head; the other, a negative inhibitory factor, the temporary physiological effect of posterior section (Child and Watanabe, 1935; Watanabe, 1935*b*). As regards head frequency, however, no decisive direct evidence has yet been given for an axial differential of either the head-determining factor or the head-inhibiting factor separately. When the inhibitory factor is eliminated by making the posterior cut at a distance sufficiently posterior to the anterior cut or by sufficient delay of posterior section, pieces of these species develop all normal heads, and no differential in head frequency is found. Moreover, data at hand do not permit us to determine whether the relation between head frequency and level of body is due to a decrease in the anterior direction of the effect of posterior section or to an increase in the anterior direction of ability to antagonize it, although the inhibitory factor is evidently effective over a greater length of piece in the posterior than in the anterior region of the anterior zooid. Now in the light of the evidence presented in this paper there can be no doubt as to the existence and form of the anteroposterior differential head-forming activity. This in turn suggests that it may be possible to obtain more definite information concerning the axial relation of the head-inhibiting factor. As regards this negative factor in this species, further data will be presented in a later paper.

The average standard deviations of mean times given in the preceding tables show, first, that variation in rate of head development increases from anterior to posterior levels of the anterior zooid (Tables 1 and 6) and, second, that at the same level it increases generally with decrease in length of piece (Table 3). These variations of mean times may be associated, at least in part, with the variations in physiological condition to which the pieces examined are subjected because of their position in the axial organization of the body and with the variations in excitation resulting from posterior section. Differences in complexity, presence of one or more than one zooid, and stage of physiological development of zooids are doubtless concerned in the variations and irregularities of mean times in the posterior zooid region.

SUMMARY

1. With a Japanese planarian known as *P. gonocephala* as material, mean times from section to appearance of eyespots were determined in pieces of equal length from different body-levels and in pieces of different lengths from the same levels. From these mean times values for relative rates of differentiation of new heads were obtained as percentages of a standard mean time taken as 100.

2. In pieces of equal length the rate of eye formation decreases from anterior to posterior levels of the anterior zooid and increases again in the posterior zooid region. This axial differential corresponds to differential susceptibility as indicated by disintegration in whole animals, but there is no corresponding head-frequency gradient; in this

species, under average normal conditions of laboratory environment, all pieces from all levels develop normal heads.

3. At the anterior level immediately posterior to the head, length of piece from 1/16 to 4/8 has little or no effect on rate of eye formation, while at more posterior levels the rate increases as length of piece increases from 1/16 to 3/8 and attains a maximal rate in 3/8 pieces for each level.

4. A transverse half-section at a very short distance posterior to the anterior cut end of the piece retards eye formation and the rate of growth of new tissue on the side of the half-section in pieces from the posterior region of the anterior zooid but is not effective in pieces from the anterior region just behind the head.

5. The maximal rate obtained in 4/8 pieces differs according to levels of body. This fact confirms, independently of the effect of length of piece, the evidence for the antero-posterior differential in rate of head development.

6. Variations in mean times of appearance of eyespots increase in general from anterior to posterior levels of the anterior zooid and with decrease in length of piece.

7. In 1/8 pieces from all levels of an aberrant stock, rate of eye formation is very high, variation small, and both nearly equal at all body-levels. In these respects the pieces from all levels of this aberrant stock resemble the pieces from the region immediately behind the head of ordinary stocks.

LITERATURE CITED

ABELOOS, M. 1927. La Vitesse de régénération de la tête chez *Planaria gonocephala* Dugés. Compt. rend. Acad. Sci. Paris, **184**:345.

———. 1930. Recherches expérimentales sur la croissance et la régénération chez les planaires. Bull. biol. France et Belgique, **64**:1.

BUCHANAN, J. W. 1933. Regeneration in *Phagocata gracilis* (Leidy). Physiol. Zoöl., **6**:185.

CHILD, C. M. 1932. Experimental studies on a Japanese planarian. I. Fission and differential susceptibility. Sci. Repts. Tohoku Imp. Univ., Biol., **6**:314.

CHILD, C. M., and WATANABE, Y. 1935. The head frequency gradient in *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:1.

FISHER, R. A., and YATES, F. 1938. Statistical tables, for biological, agricultural and medical research **8**:90. London: Oliver & Boyd, Ltd.

HYMAN, L. H. 1931. Studies on the morphology, taxonomy and distribution of North American Triclads. IV. Trans. Amer. Micros. Soc., **50**:336.

———. 1939. North American Triclad Turbellaria. IX. *Ibid.*, **58**:264.

SIVIČKIS, P. B. 1923. Studies on the physiology of reconstitution in *Planaria lata*, with a description of the species. Biol. Bull., **44**:113.

———. 1931*a*. A quantitative study of regeneration along the main axis of the triclad body. Arch. zool. ital., **16**:430.

———. 1931*b*. A quantitative study of regeneration in *Dendrocoelum lacteum*. Arb. d. II Abt. d. ungar. biol. Forschungsinstitut, **4**:1.

———. 1933. Studies on the physiology of regeneration in triclads. V. D. Univ. Mat.-Gamtos Fak. Darbai, **7**:369.

———. 1934. The effect of the regeneration activity upon the rate of regeneration of eyes in *Planaria lugubris*. *Ibid.*, **9**:13.

WATANABE, Y. 1935*a*. Rate of head development as indicated by time of appearance of eyes in the reconstitution of *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:41.

———. 1935*b*. Head frequency in *Euplanaria maculata* in relation to the nervous system. *Ibid.*, p. 371.

———. 1937. Susceptibility of planarian pieces to various concentrations of Murray-Ringer solution Sci. Repts. Tohoku Imp. Univ., Biol., **11**:279.

———. 1941. Effects of modified Ringer solution on head reconstitution in *Euplanaria dorotocephala*. Physiol. Zoöl. **14**:316.

DIFFERENTIAL MODIFICATIONS OF COELOM DEVELOPMENT IN THE STARFISH, PATIRIA MINIATA

(Thirty-two figures)

C. M. CHILD
Hopkins Marine Station, Pacific Grove, California, and
School of Biological Sciences, Stanford University

IT IS well known that modifications of form and proportions result from exposure of eggs and embryos to toxic agents and conditions. A considerable body of evidence based on experiment indicates that modifications of early development produced by particular agents are very generally not specifically different from those produced by other agents. Within a certain range of concentration or intensity of action similar modifications are produced by many different agents and conditions which unquestionably act in different ways on living protoplasms. In general, these modifications are differential in character; that is, alteration of form and proportions is not localized in a particular region or organ primordium but is graded in relation to the axiate pattern of the organism or part concerned and its changes in the course of development. The differential character of the modifications results from a differential susceptibility generally characteristic of physiological axes; and this, in turn, is associated with graded, apparently quantitative, axial difference in physiological condition, involving metabolism and substrate. The most intensely active regions are most susceptible to the direct toxic action of a large number of agents and are consequently most inhibited in development by this action, and the degree of inhibition decreases with decreasing activity along the axis. With the lower ranges of toxic action of many agents secondary developmental modifications, opposite in direction as regards form and proportion, result from differential tolerance, differential acclimation or conditioning, and differential recovery after temporary exposure. These modifications usually follow some degree of primary differential inhibition. In general, the regions most susceptible to the more extreme toxic actions show, in the course of time, greater tolerance and greater or more rapid acclimation or conditioning with continuous exposure, and recover more rapidly or more completely after temporary exposure to the less extreme actions of agents. In early development of various organisms polar, ventrodorsal, or dorsiventral patterns of the whole organism and of organ systems all show differential modifications of similar character, apparently dependent on quantitative, rather than specific, differences in condition along the axes. Axiate pattern may be completely obliterated by differential inhibition, or the regional differences along an axis may be increased beyond the normal by the secondary modifications.

Early echinoderm development shows a wide range of differential modification with many agents, both chemical and physical, apparently because specific regional differentiations along the axes have not proceeded very far in the egg and embryo. At any rate, the modifications show little evidence of specific regional differences. Certain modifications have been regarded by some investigators as indicating such differences and as specific effects of particular agents, but this is perhaps still open to question.[1]

[1] For experimental data on differential modification of echinoderm development by various agents and its physiological basis and for citations and discussion of literature see the following: Child, 1915; 1916*a*; 1916*b*; 1936*a*; 1936*b*; 1938; 1940; 1941, chap. vi.

The present paper is primarily concerned with differential modifications of coelom development, but many of the figures show differential modifications of larval form and proportions. The figures of experimentally modified development represent the most advanced stages attained by the individuals figured. It is a point of considerable interest that the greater the degree of differential inhibition the earlier the stage at which development stops. The figures are diagrammatic but are drawn from sketches of the living animals, immobilized when necessary, so that ocular micrometer measurements could be made as a basis for figures. The ciliated bands, so far as they are present, are indicated in broken lines. Parts of gut or coeloms seen through other parts of these organs are represented in dotted line. Experimental procedure in each case figured is given in the legend.

The data were obtained at the Hopkins Marine Station, Pacific Grove, California, in the winter of 1936; publication has been delayed by other work. The kindness of the director, Dr. W. K. Fisher, and of the staff in providing facilities and material is again gratefully acknowledged.

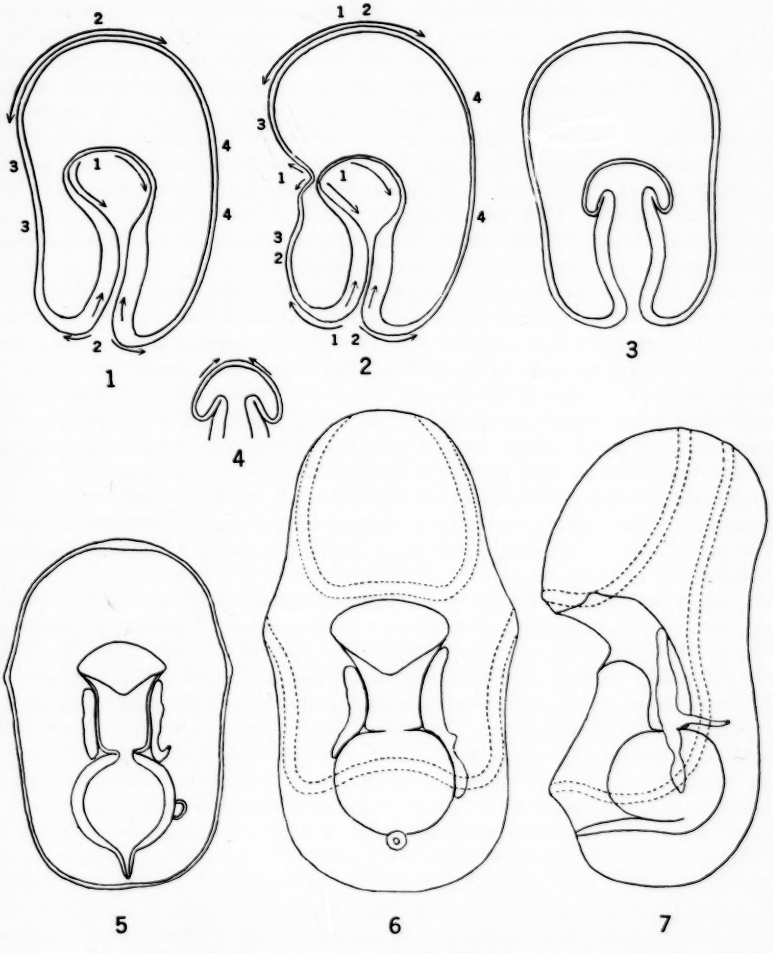
COELOM DEVELOPMENT IN *Patiria* UNDER NATURAL CONDITIONS

After invagination of entoderm is completed, the apical or anterior region of the archenteron enlarges greatly, its wall becomes thinner, and mesenchyme cells are given off from it. With these changes rate of dye reduction in low oxygen increases greatly in the enlarged region, the increase being greatest in the median anterior region (Fig. 1) and apparently somewhat greater anterodorsally than ventrally. This suggests that the enlargement and change in form of this region are associated with a considerable activation of its cells. Dye reduction becomes more rapid there than in any other part of the later gastrula except the extreme apical ectoderm in some individuals. With extension of this archenteric region ventrally to meet the stomodeal invagination the region of most rapid dye reduction appears to shift somewhat ventrally (Fig. 2). The two anterior coeloms develop as lateral budlike outgrowths extending more or less posteriorly from the enlarged archenteric region (Fig. 3). As they develop, rate of dye reduction becomes temporarily higher in them than in the median region (Fig. 4). The two coelomic buds separate completely from the entoderm, undergo elongation, and from one, somewhat more frequently the left, but often the right, the tubular madreporic canal extends dorsally to the ectoderm and opens to the exterior. The median region of the anterior archenteron becomes the foregut or esophagus (Fig. 5). During these changes a posterior coelomic bud develops from the midgut, more commonly on the left side (Fig. 5), and the anterior coelom of the same side extends posteriorly and unites with it (Figs. 6 and 7).[2]

DIFFERENTIAL MODIFICATION OF COELOM DEVELOPMENT BY LITHIUM CHLORIDE

The anterior coeloms.—The modifications of anterior coelom development show a wide range of variation in different individuals of a single lot, all in the same container and exposed to the same concentration of LiCl at the same stage and for the same time. This variation probably results in part from individual differences in susceptibility, but even without crowding there may be considerable local differences in environment in the same container. For individuals of nonmotile early stages, more or less aggregated in groups, environment as regards LiCl, oxygen, and CO_2 must become increasingly different from that of widely

[2] For studies on asymmetry in *Patiria* see Newman, 1921*a*, 1921*b*, 1922, 1925.



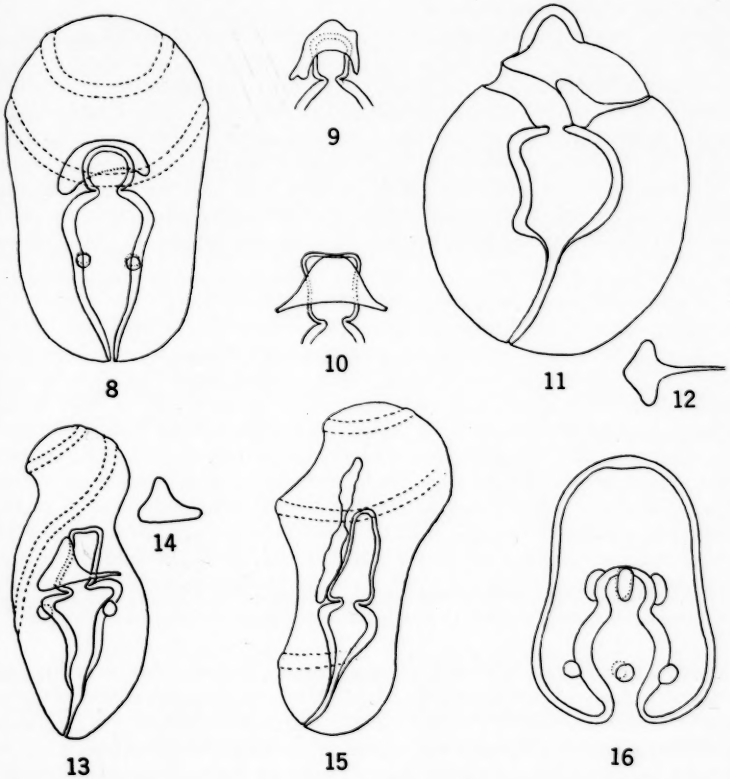
FIGS. 1–7.—Normal development. Figs. 1 and 2, differential dye reduction in gastrula stages; arrows indicate directions of progress of reduction from regions of most rapid reduction, numerals, relative times of reduction in different regions, two numerals in the same region indicating variation in time in different individuals (from Child, 1936*a*). Fig. 3, formation of anterior coeloms. Fig. 4, course of dye reduction at stage of coelom buds. Fig. 5, later stage in ventral view, showing anterior coeloms, the left one with madreporic canal, and the posterior coelom of the left side of midgut. Figs. 6 and 7, ventral and lateral outlines of advanced larvae.

scattered individuals. Aggregations of swimming stages may also alter their environment. Slight difference in physiological condition permitting individuals to swim at the water surface instead of moving about in contact with the bottom may determine considerable differences in developmental modification. Dye reduction indicates that oxygen content is normally low in the blastocoel, and with slight differences in environment it may be above or below a critical level. Renewal of solution or change of water may alter the environment of many individuals in the lot. Consequently it is not certain that the modifications in LiCl solutions are all due entirely to LiCl, and the same is true for other agents; but comparison with controls makes it sufficiently evident that the agent is the primary factor in bringing about the modifications. They are all effects—first of differential inhibition, with perhaps secondary modifications. In the lithium modifications described below the secondary modifications represent differential recovery after return to water.

The usual modification of anterior coelom development with moderate inhibition is approach of the loci of coelom development to the median plane anterodorsally on the foregut, with resulting formation of a single sac with the usual asymmetry more or less evident or reversed or with complete bilateral symmetry. With more extreme inhibition a single rounded median sac may develop at the anterior end of the foregut. From the differentially inhibited coelom a madreporic canal may or may not develop, and, if it develops, may end blindly without reaching the ectoderm; a bilaterally symmetrical single coelom often gives rise to two canals, left and right, bilaterally localized. In Figure 8, viewed ventrally, the right side of the single coelom is the larger and there is no canal; in Figure 9, a dorsal view, the left side is larger, the short blind outgrowth from it is probably an inhibited madreporic canal; the foregut is distinctly inhibited. Figure 10, a dorsal view, shows a case of bilaterality with two canals, both reaching the surface. The single coelom of Figure 11 is anterodorsal and median, somewhat bilateral in form when viewed anteriorly (Fig. 12), and the single madreporic canal is median. These modifications represent primarily results of differential inhibition of the foregut region. In the individuals of Figures 8–10 the foregut ends blindly without extending ventrally, and no stomodeum develops. The inhibition is greatest in the median anterodorsal region of the foregut, corresponding closely to the region of most rapid dye reduction in the later gastrula. The animals figured were all returned to water before gastrulation, and coelom development took place only after more or less recovery from lithium effects. Recovery, however, was not complete, and the gradient level from which coelom develops is nearer the median plane anterodorsally on each side, so that a single coelom with more or less indication of its double character, instead of two separate sacs, develops. Normally the anterodorsal side of the foregut undergoes much greater increase in size than the other side as the extension ventrally takes place (Fig. 7). Since the ventral extension does not take place in the cases of Figures 8–10, inhibition of the foregut is evidently greater anterodorsally than ventrally, and approximation and union of the regions of coelom development usually result. Even in cases like Figure 11, in which the foregut extends ventrally and connects with a small mouth, its anterodorsal side is more inhibited than the ventral, as comparison with the normal control (Fig. 7) shows.

A single anterodorsal coelom is not the only modification resulting from differential inhibition by lithium. Not infrequently a single asymmetrical or symmetrical coelom develops on the ventral side of the foregut. In the individual of Figures 13 and 14 the single ventral coelom is asymmetrical with a madreporic canal extending around the left side of the foregut and opening laterodorsally on the left side. Here the dorsiventrality of the foregut is apparently reversed; and it extends more or less dorsally in the blastocoel, that is, there is little

or no recovery dorsally and the coelom develops from the less susceptible ventral side. Another case of single ventral coelom, median and without canal, resulting from longer exposure

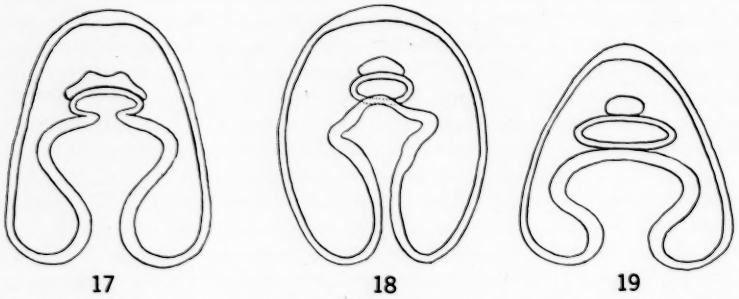


FIGS. 8–16.—Modifications resulting from exposure to LiCl for certain periods in early stages, followed by return to water. Fig. 8, LiCl, m/25, 11 hours from 32 to 64 cells, 3 days water. Fig. 9, dorsal view of asymmetrical coelom and inhibited foregut, probably an inhibited canal on left; same treatment as in Fig. 8. Fig. 10, dorsal view of bilateral coelom and inhibited foregut; same treatment as Fig. 8. Fig. 11, lateral view of differentially inhibited larva with median dorsal coelom and median canal; LiCl m/30, 21¼ hours from 2-cell stage, 7 days water. Fig. 12, coelom and canal of Fig. 11 in anterior view. Fig. 13, ventral anterior coelom with canal on left, foregut much inhibited with ventral side longer than dorsal, 2 posterior coeloms; LiCl m/25, 11 hours from 32 to 64 cells, 8 days water. Fig. 14, coelom of Fig. 13 in ventral view. Fig. 15, ventral coelom with more advanced development, no posterior coelom, 3 ciliated bands, posterior band only on ventral side; LiCl m/30, 38½ hours from 64 cells, 10 days water. Fig. 16, larva completely radial in form with 4 anterior and 4 posterior coeloms; LiCl m/25, 19½ hours from 32 to 64 cells, 4 days water.

to a somewhat lower concentration of LiCl, is shown in Figure 15. Here the differential inhibition was sufficient to reverse dorsiventrality of the foregut and localize coelom develop-

ment ventrally, but after return to water the coelom itself underwent considerable recovery. In general, ventral coelom development is more frequent with high concentrations or long exposures, but a few individuals with ventral coeloms may appear in the same lots with those developing single anterodorsal coeloms.

Occasionally three or four coeloms develop, apparently equally spaced about the foregut (Fig. 16). In these cases dorsiventrality is apparently completely obliterated in the foregut by differential inhibition, and ventrodorsality is more or less completely obliterated in the whole larva. In the larva of Figure 16 foregut and all other parts of the larval body are completely radial in form. In some of these cases, however, slight ventrodorsality of form in the ectoderm or the beginning of stomodeal invagination indicates that the coeloms are dorsal, lateral, and ventral; but in forms like Figure 16 there is no evidence of ventrodorsality. A few individuals with a single dorsal and a single ventral coelom have been seen, and two individuals with two coeloms oblique to the median plane and opposite or nearly opposite. In



FIGS. 17–19.—Modifications resulting from continuous exposure to LiCl m/40 from 24-hour blastula; 8 days LiCl; coelom completely anterior to greatly inhibited foregut and no posterior coelom.

these the foregut shows no evidence of dorsiventrality, but the larval form is slightly bilateral. These forms with three or four coeloms about a radial foregut evidently represent more or less recovery after complete or almost complete obliteration of dorsiventrality in the foregut, but how localization about the polar axis and number of coeloms are determined is not evident.

In all cases described thus far except that of Figure 15 the material was returned to sea water from LiCl before gastrulation began. At the time of return to water development was retarded, and the blastulae were somewhat smaller than the controls. With the higher concentrations and longer exposures there was more or less exogastrulation after return to water. Entogastrulation after lithium treatment resulted in forms smaller than controls with ectoderm more inhibited than entoderm, which consequently appeared relatively "too large." Enlargement of the anterior region of the archenteron was usually more or less inhibited and smaller than normal, and, as the figures show, the foregut usually remained blind with little or no ventral extension or even some extension toward the dorsal side (Figs. 13 and 15), and stomodeal development was completely inhibited; the more inhibited individuals often remained completely radial (Fig. 16).

With continuous exposure to concentrations of LiCl, not lethal before development stops but producing distinct differential inhibition, forms like Figures 17–19 result. Morphological ectodermal development does not proceed beyond the earlier gastrula stages, and ectodermal

inhibition decreases from the apical region basipetally. The entoderm is relatively "too large" but usually elongates less, and the anterior enlargement is smaller than in controls and at best does not develop beyond the stage of a rounded or ovoid sac, often apparently no longer open into the midgut (Figs. 18 and 19). The anterior coelom is usually single without any trace of asymmetry and almost or entirely anterior to the foregut. It may be partly double, as in Figure 17, or merely slightly elongated laterally (Figs. 18 and 19) and does not develop further. Coelom development in these cases may represent secondary modification involving some degree of differential tolerance or conditioning to LiCl.

With more extreme inhibitions the anterior coelom may lose epithelial character at an early stage and dissociate into free cells like mesenchyme, or the whole anterior archenteron may undergo more or less complete dissociation before coelom development; more or less dissociation of entodermal cells into the blastocoel may occur during, or even before, gastrulation, the latter case resembling immigration of mesenchyme in echinoids (Child, 1940).

The posterior coelom.—The usual effect on posterior coelom development of moderate inhibition by temporary exposure to LiCl is development of two, three, or more posterior coelomic buds at the same level about the midgut (Figs. 8, 13, and 16). In one individual six of these buds were present, equally spaced. With increase in number of posterior coeloms evidence of asymmetry is usually absent, even though it may be present in anterior coelomic development (Fig. 8). The modifications suggest that some degree of dominance of one side, usually the left, is present in the uninhibited animal and that under inhibiting conditions this dominance is obliterated. Longer exposures to concentrations which still permit anterior coelomic development after return to water usually inhibit posterior coelom development completely (Figs. 11 and 15), that is, the anterior entodermal region recovers more completely than the midgut. With continuous exposure to inhibiting concentrations permitting some degree of anterior coelomic development there is usually no posterior coelomic development (Figs. 17–19), the midgut apparently never attaining the stage at which this development becomes possible.

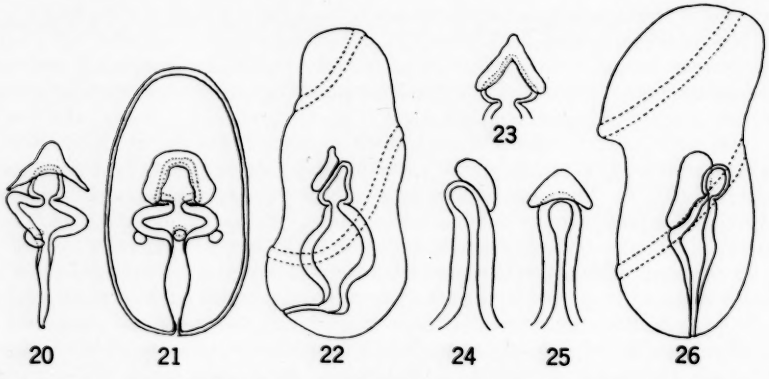
DIFFERENTIAL MODIFICATION OF COELOM DEVELOPMENT BY OTHER AGENTS

In the course of experiments on differential modification of development by other agents it was found that the same series of modifications of coelom development appear as with LiCl.

Nile blue sulphate.—In order to determine whether or to what extent Nile blue sulphate might be toxic and produce differential inhibition of echinoderm development, it was used in very low concentrations, which stained the embryos and larvae deeply. One part in ten thousand in sea water did not dissolve completely or precipitated in part. Various dilutions of the part in solution were used; but, within a wide range, difference in concentration makes no great difference, for the dye is accumulated in the cells even from highly dilute solutions. In the higher concentrations it becomes toxic somewhat earlier because it accumulates more rapidly. Coelomic modifications are like those produced by LiCl. Union anterodorsally of the two anterior coeloms with more or less complete disappearance of asymmetry and failure of the foregut to extend ventrally are the most frequent. With more extreme effects a single entirely anterior coelom or a ventral coelom develops. Two, three, or four posterior coelomic buds appear frequently. Figures 20–23 show a few characteristic modifications. In all these cases the foregut is evidently inhibited, does not extend ventrally, and stomodeum is absent. In Figure 20 the single anterior coelom is bifurcated with two symmetrical canals and the

posterior coelom is elongated, extending about halfway around the gut, probably in consequence of continuity of two bud loci. In Figure 21 the posterior portions of the anterior coelom extend ventrally almost around the inhibited foregut, but canals are entirely absent; four posterior coelom buds are present. Except for the bilaterality of the anterior coelom and perhaps the foregut, the larva is radial in form. The single anterior coelom of Figure 22 is ventral (Fig. 22), but bilateral (Fig. 23) and posterior coelom development is completely inhibited. These three individuals are from the same lot; for other gradations of modification the figures for LiCl will serve as well as any.

Neutral red.—Lots kept for 2 days in very low concentrations of neutral red with gradual further dilution by precipitation show the same series of coelomic modifications: single



FIGS. 20–26.—Modifications by Nile blue sulphate and neutral red. Figs. 20–23, Nile blue sulphate, saturated (?) sea-water solution diluted 1/2, 19 hours from 32 to 64 cells, 5 days water. Fig. 20, dorsal outline of bilaterally symmetrical anterior coelom and gut, foregut greatly inhibited, blind, without ventral extension, no stomodeum, posterior coelom partly surrounding midgut; Fig. 21, dorsal view, anterior coelom bilateral, partly surrounding inhibited, almost radial foregut, no stomodeum, 4 posterior coeloms; Fig. 22, larva with ventral coelom; Fig. 23, coelom and foregut of Fig. 22 in ventral view. Figs. 24–26, 2 days weak neutral red from 19-hour blastula, 4 days water. Figs. 24 and 25, anterodorsal coeloms with inhibited gut; Fig. 26, a ventral coelom somewhat on the right side.

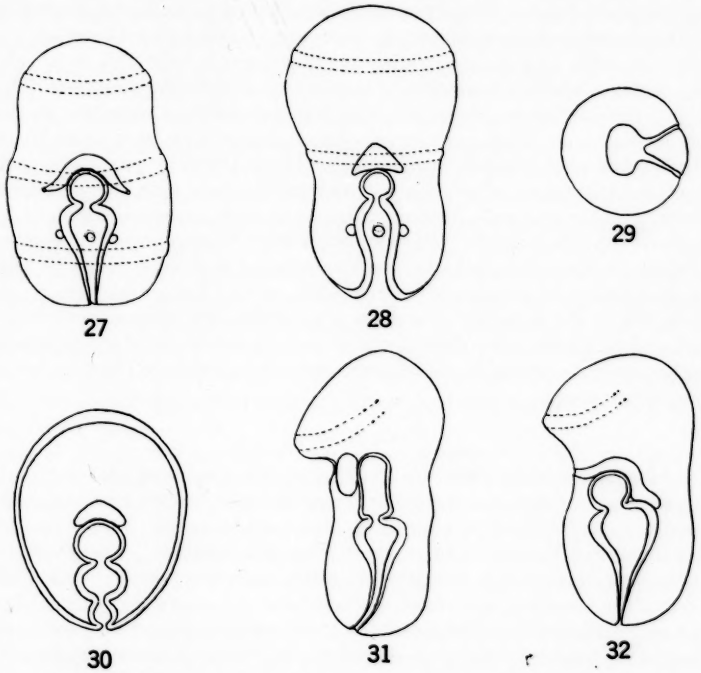
asymmetrical or bilateral anterior coeloms (Figs. 24, 25) and some cases of ventral coelom (Fig. 26). The entoderm in these forms is often much inhibited, as in the figures, probably because it stains deeply; anterior coelom development appears to be a secondary modification of the anterior entodermal region following return to water. The general form of the larva often shows distinct evidence of secondary modification, the preoral region becoming much enlarged and the anterior coelom relatively very large, as compared with the gut. The animals apparently are able gradually to dispose of some of the dye in some way if not too much injured at first, and this process is also differential. Posterior coeloms have not been seen in any of the neutral red modifications; the more posterior regions of the entoderm are apparently not able to dispose of the dye as rapidly as the anterior region.

Acidified sea water.—Acidification to pH 6.6 by HCl also modifies coelom development in the same ways as LiCl. The effective agent in this case may be CO_2 rather than hydrogen ion.

Hypotonic sea water.—Eighty per cent sea water gives a high frequency of coelomic modi-

fications, primarily differential inhibitions with more or less secondary modification in some cases and indistinguishable from the LiCl modifications. In 90 per cent sea water the modifications are, in general, less extreme and less frequent.

Tobacco smoke.—This was found to be extremely effective in producing differential inhibition. A dry finger bowl covered immediately and left standing for a day after smoke is



FIGS. 27–32.—Smoke. Figs. 27 and 28, bilateral anterior coeloms, 4 posterior coeloms; Fig. 29, anterior view of coelom of Fig. 28; Fig. 30, coelom entirely anterior to inhibited foregut, larva entirely radial except for slight bilaterality of coelom, no posterior coelom; Fig. 31, median ventral coelom between stomodeal invagination and inhibited foregut, no posterior coelom; Fig. 32, coelom median, anterodorsal, opening with ventral canal and pore. These animals were in smoked finger bowls during early development with one or more changes of water shortly before or in early gastrulation.

puffed into it makes sea water differentially inhibitory. Even finger bowls kept after washing in a room in which there was considerable smoking produced differential inhibition of coelom development and of larval form. Coelomic modifications show the same series as with LiCl and other agents. Figures 27–32 are examples. The larva of Figure 27 is completely radial in form with three ciliated bands extending horizontally around the body, a common result of differential inhibition. The foregut is merely a rounded sac, but the anterior coelom is bilateral with two canals, the only evidence of bilaterality in the larva. Four posterior coeloms are present. In the animal of Figures 28 and 29, also radial in form, the anterior coelom is

entirely anterior to the small inhibited foregut, and two canals are present symmetrically localized near the median plane (Fig. 29). This individual also has four posterior coeloms. Figure 30 shows a more extreme inhibition with slight secondary modification of the anterior region of the archenteron following changes of water after the inhibition by products of smoke. In Figure 31 the coelom is ventral, lying between the foregut and a slight stomodeal invagination. The single median anterior coelom of Figure 32 opens in the median ventral region, and the stomodeum is completely inhibited. Ventral pores have been seen occasionally with other agents but never frequently under any conditions used thus far.

Old stock.—Animals kept 9 days in a laboratory aquarium before use were very slow in discharging eggs and sperm when removed from water, and development was differentially inhibited, with the same series of coelomic modifications as with other inhibiting conditions.

Complete inhibition of coelom development.—Development may be stopped at practically any stage according to degree and rapidity of inhibiting action by any of the agents mentioned above and by various others. Entodermal modifications with complete inhibition of coelom development constitute a further graded series with increasing degree of inhibiting action. More or less enlargement of the anterior archenteron may occur but never reach the stage of coelom development, and the entoderm remains without evidence of bilaterality. The enlargement may be completely inhibited, the wall of the anterior region remaining thick like the rest of the entoderm, and there is no visible difference or boundary between foregut and midgut. More or less dissociation of the anterior region of the archenteron may occur after or during invagination, or cells may even dissociate from the entodermal region before invagination begins.

DISCUSSION

The inhibiting action on the entoderm of all agents and conditions used is very distinctly differential, the anterior region of the archenteron, which normally undergoes enlargement and apparently marked activation, being most inhibited. Moreover, even in this region the inhibition is differential, the anterodorsal region being most inhibited. The differential modifications of coelom development are physiologically essentially similar to the differential modifications of the planarian and vertebrate head and the sea-urchin pluteus. In the planarian head under inhibiting conditions all degrees of approximation to the median plane of eyespots and of cephalic lobes and corresponding obliteration of median regions of the cephalic ganglia appear, according to degree of inhibiting action.[3] With differential inhibition of the head in fishes and amphibia there is a similar progressive approximation and union in the median plane of loci of development of bilateral cephalic organs as inhibiting action increases.[4] In the differentially inhibited sea-urchin larva the anal arms are approximated to the median plane, a single median arm may develop, or arm development may be completely inhibited and bilaterality obliterated.[5] Evidently, all these modifications result from differential susceptibility to inhibiting agents and conditions, depending on differences in physiological condition which are graded rather than sharply defined or localized. Since these differences in susceptibility in the planarian and vertebrate head, the sea-urchin larva, and the starfish archenteron are not specifically different for different agents, the conclusion appears justified that they are associated with, and depend on, quantitative differences in

[3] Child, 1921; Child and McKie, 1911; Child and Watanabe, 1935.

[4] See, e.g., Stockard, 1907, 1909; Bellamy, 1919; Child, 1941, chap. vii.

[5] Child, 1916*b*, 1938, 1940*a*, 1941.

physiological condition rather than on qualitative differences in constitution, even though such differences may be present in greater or less degree. If they are present, they are apparently not the factors concerned in these differential modifications.

Development of a single coelom on the ventral side of the foregut apparently results from inhibition of lateral, dorsal, and anterior regions to such a degree that they cannot give rise to coeloms. After return to water or after repeated change of water in the case of smoke, dorsal and anterior regions do not recover sufficiently before development stops to develop a coelom, but the less susceptible ventral side is sometimes not too much inhibited to develop a coelom. Development of three or four coeloms about the foregut probably results from obliteration of dorsiventrality; there is no evidence of dorsiventral difference in such cases.

From the modifications it is evident that anterior coelom development can take place from lateral, dorsal, anterior, or ventral regions of the foregut and that the number of coeloms may range from one, without or with evidence of bilaterality or asymmetry, to four. Evidently the locus of coelom development is not a fixed, specifically determined region but depends on the gradient in physiological condition in the anterior part of the archenteron and foregut and on the alterations of this gradient by external factors. The median anterior and anterodorsal region of the anterior archenteron represent the high region of the gradient system, and coelom development is localized in a region on each side defined by the gradient system. Under inhibiting conditions the gradient level for coelom development is shifted nearer the median plane anterodorsally or anteriorly or, with certain degrees of inhibition, to the ventral side; and with obliteration of dorsiventrality the apicobasal gradient in the anterior archenteron is the only localizing factor remaining, and several coeloms may develop at the same level about the foregut.

The asymmetrical position of the posterior coelom, usually on the left, and the appearance of two, three, four, and in one case six coelom buds about the midgut, suggests that one side is usually dominant under natural conditions and that with obliteration of asymmetry this dominance is abolished. That one side develops more rapidly and inhibits the other was suggested by Newman (1925). The frequent development of equal paired madreporic canals from differentially inhibited anterior coeloms may result from a similar elimination of dominance of one side over the other.

In view of the hypothesis that lithium has a specific action in enhancing, extending, or in some way making more effective a vegetal concentration gradient of substance (Runnström), it is a point of particular interest that the modifications of development produced by LiCl do not differ in any characteristic manner, so far as could be determined from hundreds of preparations, from those produced by other agents. They do not provide any evidence for specific action of lithium on morphological entodermal or coelomic development. At the time of its enlargement and apparent activation the anterior archenteric region is differentially inhibited in the same way as by other agents, and the same series of developmental modifications results. In short, whatever the constitution of this region, the morphological modifications of development by LiCl and other agents thus far used apparently depend on quantitative physiological differentials rather than on localizations of specific substances or metabolisms.

SUMMARY

1. With LiCl and various other agents which inhibit development similar series of modification of localization and development of the coeloms appear in the starfish, *Patiria miniata*.

2. The modifications of anterior coelom development consist in approximation to the median plane anterodorsally of the loci of coelom origin with more or less complete union of

right and left coelom, decrease or obliteration of asymmetry, a single bilateral coelom with two madreporic canals or with none; with certain degrees of inhibition followed by some recovery, a single coelom ventral to the foregut; with obliteration of dorsiventrality in the foregut followed by recovery, development of three or four coelom buds about the foregut; with continuous exposure to certain degrees of inhibition, completely radial larval form and a single coelom entirely anterior to the greatly inhibited, completely radial foregut. With still more extreme inhibition anterior coelom development is completely inhibited, and dissociation of cells from the apical archenteron or from the whole entodermal region may take place.

3. Modifications of posterior coelom development by differential inhibition consist in obliteration of the normal asymmetry; and with recovery after return to water two, three, four, in one case six, posterior coelom buds develop at the same level about the midgut. With more extreme inhibition posterior coelom development is completely inhibited. The midgut region is less capable of recovery after return to water than the foregut.

4. The modifications result from graded differential inhibitions and differential recoveries, not from specific local actions of agents. Since they are not specific for LiCl or any other agent used, they are believed to depend on quantitative differentials in physiological condition, and presence of such differentials is indicated by other methods.

LITERATURE CITED

BELLAMY, A. W. 1919. Differential susceptibility as a basis for modification and control of development in the frog. I. Biol. Bull., **37**:312.

CHILD, C. M. 1915. Axial gradients in the early development of the starfish. Amer. Jour. Physiol., **37**:203.

———. 1916*a*. Axial susceptibility gradients in the early development of the sea urchin. Biol. Bull., **30**:391.

———. 1916*b*. Experimental control and modification of larval development in the sea urchin in relation to the axial gradients. Jour. Morph., **28**:65.

———. 1921. Studies on the dynamics of morphogenesis. XI. Jour. Exper. Zoöl., **33**:409.

———. 1936*a*. Differential reduction of vital dyes in the early development of echinoderms. Arch. f. Entw'mech., **135**:426.

———. 1936*b*. A contribution to the physiology of exogastrulation in echinoderms. *Ibid.*, p. 457.

———. 1938. Certain differential modifications of asteroid and echinoid development. Annot. Zool. Japon., **17**:205.

———. 1940. Lithium and echinoderm exogastrulation, etc. Physiol. Zoöl., **13**:4.

———. 1941. Patterns and problems of development. Chicago: University of Chicago Press.

CHILD, C. M., and MCKIE, E. V. M. 1911. The central nervous system in teratophthalmic and teratomorphic forms of *Planaria dorotocephala*. Biol. Bull., **22**:39.

CHILD, C. M., and WATANABE, Y. 1935. The head frequency gradient in *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:1.

NEWMAN, H. H. 1921*a*. On the occurrence of paired madreporic pores and pore canals in the advanced bipinnaria larva of *Asterina* (*Patiria*) *miniata*, etc. Biol. Bull., **40**:118.

———. 1921*b*. The experimental production of twins and double monsters in the larvae of the starfish *Patiria miniata*, etc. Jour. Exper. Zoöl., **33**:321.

———. 1922. Normal versus subnormal development in *Patiria miniata*. Biol. Bull., **43**:1.

———. 1925. An experimental analysis of asymmetry in the starfish, *Patiria miniata*. *Ibid.*, **49**:111.

STOCKARD, C. R. 1907. The artificial production of a single median cyclopian eye in the fish embryo by means of sea water solutions of magnesium chloride. Arch. f. Entw'mech., **23**:249.

———. 1909. The development of artificially produced cyclopian fish. "The magnesium embryo." Jour. Exper. Zoöl., **6**:285.

THE ALTERATION OF DEVELOPMENTAL PATTERN IN THE SAND DOLLAR BY PILOCARPINE[1]

(Forty-three figures)

OLIN RULON
Hopkins Marine Station and Wayne University

OTHER work on the dynamics of echinoderm development (Child, 1936*a*, 1936*b*, 1940; Rulon, 1938, 1941) has shown that such agents as lithium and sodium thiocyanate affect early developmental pattern primarily through inhibitory action. The inhibition of certain active regions may be followed secondarily by recovery effects, depending upon the experiment, to produce modifications of widely different types. Differential conditioning may also take place in low concentrations. By recognizing differential inhibition, recovery, and conditioning in the modified embryo it is possible to tell something of the time of origin, the localization of particular fields, the development of symmetry, and other factors operating in the progressive development of the normal pattern.

With data on the effects of inhibitory action available, it seemed desirable to test the effect on developmental patterns of an agent which reputedly has a stimulating action. Since there were many indications that pilocarpine might fill this need, a number of experiments were performed with this substance as the test agent.

MATERIAL AND METHODS

The eggs used in these experiments were taken from the ripe females of the sand dollar, *Dendraster excentricus*, collected near Pacific Grove, California, during the height of the breeding season. Fertilization was accomplished by adding sperm suspension and was usually 95–100 per cent effective. The solutions of pilocarpine hydrochloride were made up directly in sea water. Approximately 200 eggs were exposed to 150 cc. of solution. All cultures were kept in covered finger bowls on a water table and examined at regular intervals. Parallel control experiments were always run in normal sea water.

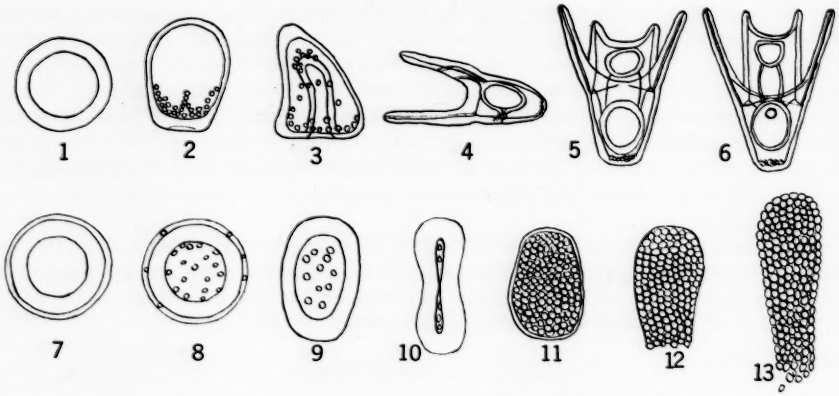
NORMAL DEVELOPMENT

For comparative purposes a few of the more important stages or landmarks in the normal development are here described. Fuller accounts may be found elsewhere (Child, 1940; Rulon, 1941).

Cleavage is total and by 7–8 hours after fertilization at the temperatures used in these experiments reaches the middle blastula stage (Fig. 1). Here the larvae have fairly thick walls, little apicobasal differentiation, and are beginning to show ciliary movement. By 12 hours after fertilization the larvae are top-swimming, the walls, with the exception of the vegetal region, are much thinner, mesenchyme has appeared in the basal

[1] The writer wishes to express his gratitude to Dr. W. K. Fisher, director of the Hopkins Marine Station, Pacific Grove, California, for the use of the laboratory space and facilities which made this work possible.

region, and there may be indications of gastrulation (Fig. 2). At 24 hours the larvae are definitely bilateral with a flattened ventral and a rounded dorsal ectoderm (Fig. 3). The archenteron has grown considerably by this time, and its apical end is directed toward the future stomodeal region. The earliest indications of anal arms may be found at the 24-hour stage where they appear as slight lateral protuberances on the posterior or anal regions of the ventral surface. The oral lobe is also turning ventrally at this stage. At 48 hours the larva has attained the middle pluteus stage with long anal arms, a definite skeleton, stomodeum, flattened oral lobe, and short oral arms (Fig. 4, lateral; Fig. 5, anal). At 60 hours the oral lobe is narrower and the arms longer (Fig. 6). Development usually stops between 60 and 70 hours after fertilization, and the larvae begin to die.



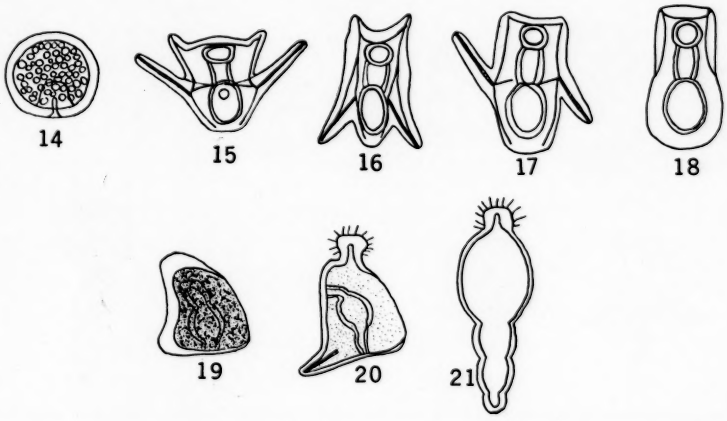
FIGS. 1–13.—Figs. 1–6, normal development of the sand-dollar larva from 7 to 60 hours after fertilization at a temperature of approximately 15° C. Figs. 7–13, inhibited larvae after 12–48 hours in M/100 pilocarpine.

EXPERIMENTAL

1. *The modification of pattern with high concentrations applied immediately after fertilization.*—When fertilized eggs were placed directly in M/100 pilocarpine and the developing larvae examined at 12-hour intervals, a clear picture of the effects of continuous exposure to this concentration was given. At the end of the first 12 hours the larvae appeared as slow-moving, thick-walled blastulae still inside their fertilization membranes (Fig. 7). The cells of these forms seemed larger than normal and showed little or none of the apicobasal differentiation that appeared in the normal blastula of this age (Fig. 2). In many, cells were seen escaping into the blastocoel from the whole of the internal lining, indicating an anaxiate condition (Fig. 8). After 24 hours in this solution the larvae were usually elongate, thick-walled forms, considerably flattened and partly or entirely filled with large cells (Figs. 9–10). A number were giving off cells to the outside from the vegetal region, while in a few there were indications of gastrulation. All were actively moving, although usually near the bottom of the culture dishes. At 36 hours they appeared chiefly as thin-walled bags of cells (Fig. 11) or as bags with cells escaping from the vegetal end (Fig. 12). At 48 hours the larvae were, for the most part, curious, elon-

gate bottom forms, filled with large cells and with cells protruding from the vegetal region (Fig. 13). They were often quite active and gave the appearance of wriggling about. At 72 hours most of the larvae had disintegrated by a progressive dissociation into separate cells from the vegetal region.

When the larvae were returned to normal sea water after the first 12 hours in M/100 pilocarpine, the developmental history was very different. After 12 hours in sea water these larvae were usually free-swimming, early gastrulae with an excess of internal cells (Fig. 14). At 60 hours a number appeared almost normal, but in many the oral and anal arms were at widened angles (Fig. 15). In others the anal arms were at aberrant angles, askew, or absent (Figs. 16–18). Some extremely inhibited forms, ranging from cell-filled blastulae to inhibited gastrulae (Fig. 19), were found near the bottom of the culture. In



FIGS. 14–21.—Larvae developing in normal sea water after an initial exposure of 12 hours to M/100 pilocarpine.

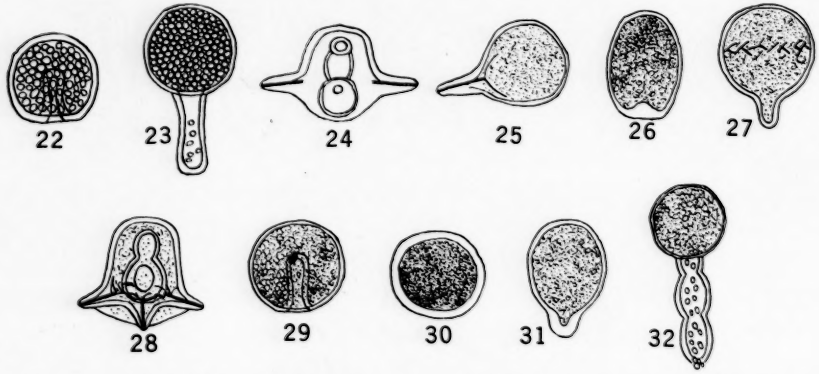
many of the inhibited forms a ciliated knob appeared at the apical region (Fig. 20). This knob—a common feature of apical differential recovery—was a rudimentary oral lobe. A few exogastrulae appeared with this treatment, and some of these had also developed a ciliated knob (Fig. 21).

Treatment for 24 hours with M/100 pilocarpine followed by return to normal sea water for the remainder of the larval period yielded forms which showed considerably more inhibition. After 24 hours in normal sea water following treatment most of the larvae appeared as free-swimming, radially symmetrical forms, filled with large cells, and showing all degrees of gastrulation and exogastrulation (Figs. 22–23). Twenty-four hours later they had reached their maximum development. There were no normal plutei, and bilaterality was evident in only a few. Many were little more than bags of cells, but a number showed a bit of skeleton and short anal arms (Fig. 24). None showed differentiation of the oral lobe, but a number possessed a gut although filled with cells and cellular debris. In some the anal arms were at aberrant angles or askew, while in others only a

single arm developed (Fig. 25). A number of the larvae were completely radial (Figs. 26–28), and, in some, short anal arms developed around the entire circumference (Fig. 28).

Treatment for the first 48 hours usually produced larvae which failed to develop either bilaterality or skeleton when returned to sea water. All were filled with cells or cellular debris, although many showed some invagination (Fig. 29). Some developed no further than blastulae (Fig. 30), while others showed an evagination, underdeveloped entoderm (Fig. 31). In a number an evaginated gut was distinctly overdeveloped (Fig. 32).

2. *The modification of pattern with medium concentrations applied immediately after fertilization.*—When fertilized eggs were exposed continuously to M/500 pilocarpine, the effects were less drastic than in the higher concentration. In the early stages this concentration had a stimulating effect on rate of cleavage, but by 11–12 hours the test

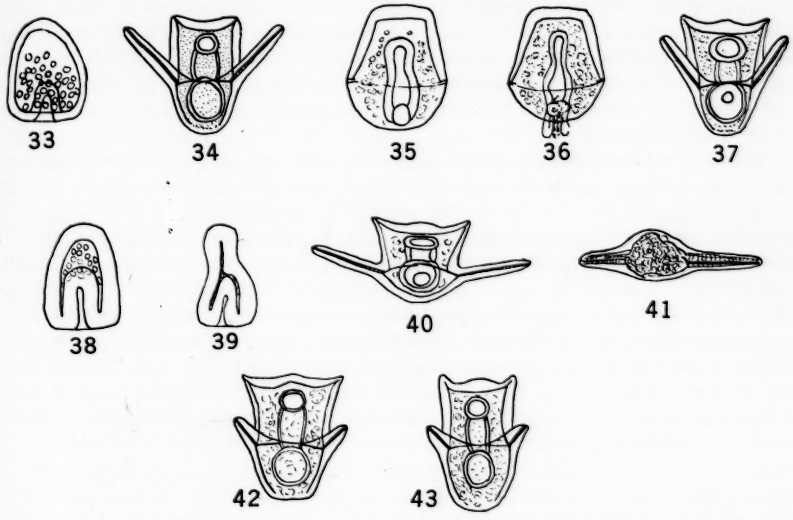


FIGS. 22–32.—Larvae developing in normal sea water after an initial exposure of 24–48 hours to M/100 pilocarpine.

blastulae were definitely inhibited in that the walls were thicker and there was less ciliary action than in the controls. An excess of mesenchyme was also found in these forms. At 24 hours in M/500 pilocarpine the larvae were chiefly top-swimming, early gastrulae (Fig. 33), which were less advanced than the controls and had noticeably thicker walls. Occasionally there was a form which had failed to gastrulate, and near the bottom of the cultures were found a number of cell-filled blastulae. After 48 hours in the test solution most of the larvae appeared as thin-walled bags of cells (Fig. 11), although in some there was a slight indication of gastrulation. It was noted in many cases that the invaginated gut became dissociated into separate cells with continued exposure to this agent. Bilaterality was also inhibited. After 72 hours most of the larvae were dead, and those that remained alive were little more than radial or irregular bags of cells. Some still had a trace of undissociated gut. Development in this solution proceeded no further, and death usually resulted by 96 hours.

When the larvae were returned to normal sea water after the first 11–12 hours in M/500 pilocarpine, the later development was not very different from that of the controls. After 60 hours in sea water most of the larvae approached normal plutei. In a

number, however, the anal arms were askew, asymmetrical, or at widened angles. A number showed an excess of mesenchyme. In some the oral lobe was inhibited, while in others the oral and anal arms were both inhibited. When the larvae were returned to sea water after the first 24 hours the effects of the agent were more noticeable. After 48 hours in sea water these forms, in general, approached the pluteus stage but presented many evidences of inhibition and recovery. In most, the oral lobes had differentiated but little, and the anal arms were at widened angles (Fig. 34). In some the anal arms



FIGS. 33–43.—Fig. 33, larva after first 24 hours in M/500 pilocarpine. Fig. 34, larva developing in normal sea water after 24 hours' initial exposure to M/100 pilocarpine. Figs. 35–36, larvae developing in M/1,000 pilocarpine. Fig. 37, larva developing in M/2,500 pilocarpine. Figs. 38–39, larvae resulting from 12½ hours' exposure to M/100 pilocarpine beginning at the middle blastula stage. Figs. 40–41, larvae resulting from 12½ hours' exposure to M/100 beginning at the middle blastula stage and a return to sea water for 48 hours. Figs. 42–43, larvae resulting from continuous exposure to M/1,000 pilocarpine beginning at the late blastula stage.

were asymmetrical or askew. Others were extremely inhibited, and all showed an excess of mesenchyme. A number showed the ciliated apical knob found in treatment with higher concentrations (Fig. 20). Forty-eight hours' exposure followed by return to sea water resulted in larvae which had undergone little differentiation. Most of the forms remained almost or quite spherical and so heavily filled with cells and internal debris that it was next to impossible to see any internal structures. In some, however, there was a trace of bilaterality, and in a few a bit of gut was seen.

3. *The modification of pattern with low concentrations applied immediately after fertilization.*—Continuous exposure to M/1,000 pilocarpine resulted chiefly in swollen forms with an excess of internal cells. These larvae had definite bilaterality but no anal or oral arms. Occasionally a trace of skeleton was seen in the anal-arm region (Fig. 35). A number

showed a portion of the gut evaginated from the anal region (Fig. 36). Although extensive differentiation was lacking, the oral lobe was larger than in the controls, and the animal as a whole presented a more flattened appearance. Continuous exposure to M/2,500 pilocarpine gave chiefly swollen forms with a widened oral lobe. Excess mesenchyme was present, and there was usually no development of oral arms. The anal arms were shorter than in the control and often were at widened angles (Fig. 37). Many normal plutei developed under continuous exposure to M/5,000 pilocarpine. In a number, however, the oral lobe was broader than normal. Practically all had well-developed oral arms which often extended at an angle from the oral lobe. Most of the larvae developing in this concentration were top-swimming as compared to the bottom forms of higher concentrations. In general, when larvae were removed from these low concentrations of pilocarpine to sea water, development proceeded toward the normal pluteus. In many cases, however, the larvae showed the effect of the agent in a widened oral lobe and shortened oral and anal arms.

4. *The modification of pattern with various concentrations applied at later stages in the developmental history.*—After 12 hours in M/100 pilocarpine, larvae which had been placed in this agent in the middle blastula stage (Fig. 1) appeared chiefly as large, active, thick-walled, cell-filled, flattened gastrulae (Figs. 38, 39). When returned to normal sea water they developed after 48 hours into forms showing various degrees of inhibition and recovery. In most of them, anal arms were present, although they ranged from extremely long structures to mere stumps. These arms were commonly at widened angles, askew, or duplicated. In most of them the oral lobe was not developed, but in some it had undergone considerable development and increase in width (Fig. 40). Occasionally a ciliated apical knob appeared. Most forms had an excess of mesenchyme, and a number had excess skeleton (Fig. 41), although in some the skeleton was diminished or lacking. Most showed evidence of bilaterality, but others seemed to be entirely radial.

Continuous exposure to M/1,000 pilocarpine beginning with the 12-hour form (Fig. 2) permitted considerable development by 70 hours (Figs. 42, 43). Almost all had definite bilaterality. An excess of internal cells was evident, and the larvae were somewhat flattened and swollen. The majority had short anal arms and an enlarged oral lobe which often had definite oral arms. The skeleton was more or less developed, and gut was usually present. When returned to sea water after 40 hours' exposure to the pilocarpine, the larvae developed very much as with continuous exposure except that the oral and anal arms became slightly longer.

DISCUSSION

It cannot be emphasized too strongly that these, or any, experiments on the modification of development must have as their aim an attempt at a better understanding of the normal pattern. The dynamics of development are extremely obscure, and anything but the broadest assumptions must necessarily at this time be tentative. However, it seems evident that the developmental modifications representing differential inhibition, differential conditioning, and differential recovery are primarily expressions of dynamic factors of developmental pattern with graded axial differentials, undergoing definite progressive change in the course of development, but with little or no evidence of regional specificity (Child, 1940, and earlier papers).

In general the effects of pilocarpine parallel the effects of other inhibitory agents, al-

though there is a slight acceleration of cleavage in low concentrations. It is not to be expected that all inhibitory agents will have an exactly similar effect. Some agents may exert a rapid action and rapidly be broken down or inactivated by the living protoplasm. Others act more slowly. Some (e.g., KCN) may exert their inhibitory action through the inactivation of respiratory enzymes, while others act in other ways. It may be that no two agents have exactly the same effect, but in general it is found that, regardless of the nature of the agent, the more active (physiologically) regions are more inhibited than the less active in high concentrations, become conditioned to inhibitory agents more rapidly in low concentrations, and recover (unless badly injured) more quickly and completely when the agent is removed.

The first noticeable effects of pilocarpine on the developing larva are the thickened wall of the blastula and an excessive number of internal cells (Figs. 8, 9, 14, 22). In low concentrations these internal cells are seen to come from the vegetal region of the blastocoel lining, and the increased number may be related to a greater susceptibility of this region at this particular stage of development. Child (1936*a*) showed with the aid of vital dyes a region of rapid reduction at this site in the normal larva at the time of formation of primary mesenchyme and entoderm. With high concentrations of pilocarpine internal cells become dissociated from the whole of the lining of the blastocoel and with continuous exposure the entire larva usually assumes the appearance of a cell-filled bag with free cells escaping externally from the vegetal region (Fig. 13). Such forms certainly approach an anaxiate condition, although at least partial return of the primary axis may take place on return to sea water (Figs. 31, 32). The cause of dissociation of cells externally from the vegetal region is not entirely clear. It may be related to an activation of that region or to a surface effect of pilocarpine which produces greater dissociation basally because the cells are slower there in attaining complete epithelial form.

With continuous exposure following fertilization the internal cells form little or no skeleton in concentrations of from M/100 to M/1,000. If returned to sea water after an exposure of 12–36 hours following fertilization, there may be sufficient recovery to form some skeleton. Often the skeletal pattern is aberrant and incomplete (Figs. 16, 17, 24, 25, 28). This may be because of a more or less complete obliteration of orienting factors in the ectoderm, which is much inhibited by these concentrations, and the pattern partly or wholly destroyed through direct inhibition or altered by irregular recovery. Anomalous distribution of mesenchymal elements, related to contact with substratum or nearness of other larvae, may also be a factor in the distortion of pattern. An excess of skeleton may develop, however, if 12-hour larvae are exposed for a short time to pilocarpine (Fig. 41). The increase in skeletal elements in this case may be the result of differential recovery of the activated mesenchyme following return to sea water.

These experiments have shown ventrodorsality to be relatively stable. In general, it develops and persists in M/1,000 pilocarpine—a concentration which, on continuous exposure, prevents or markedly inhibits the development of skeleton or oral and anal arms (Figs. 35, 36). Ventrodorsality can, however, be obliterated with continuous or short-time exposure to higher concentrations (Figs. 11, 12, 13, 26, 27, 28, 29, 30, 31, 32). Short-time exposures, immediately after fertilization, may prevent the development of ventrodorsality, yet permit the development of skeleton and anal arms (Fig. 28). Child (1936*a*) showed with vital dyes that the ventral side is the more active in a ventrodorsal gradient,

and in his experiments with lithium (1940) he noted that ventrodorsality may be completely obliterated if the larvae are treated in the early stages. The higher concentrations of pilocarpine give essentially similar results. With somewhat less extreme differential inhibition the ventrodorsality becomes evident, but the ventral side remains flat or may become convex instead of becoming deeply concave as in normal development and the arm angle is wide, even approaching 180° (Figs. 15, 24, 34, 35, 36, 37, 40, 42, 43). Some of these forms probably represent a degree of differential recovery of ventral side and mesenchyme (Figs. 15, 24, 34, 40), while others represent a degree of differential conditioning (Figs. 35, 36, 37, 42, 43).

The polar gradient is extremely stable. Concentrations of pilocarpine which inhibit the development of ventrodorsality and prevent all differentiation (except cilia formation, etc.) do not, in general, completely destroy the apicobasal gradient pattern. This is shown by eggs which have been in M/100 for 48 hours or more. On return to sea water the basal region often differentiates into entoderm, which may become much enlarged through recovery (Fig. 32). Such exogastrulae, however, fail to show any differentiation of the ectoderm. Evidences of the original polar-activity gradient are also shown in the development of the ciliated knob through differential recovery of the apical end of larvae returned to sea water following exposure to high concentrations. Such a knob (rudimentary oral lobe) indicates a region of high activity near the apical end which can recover and develop when the inhibitory conditions are removed.

In general the apical region or oral lobe is highly susceptible in the early larva and is easily inhibited by various agents including pilocarpine. The appearance of the ciliated knob in larvae treated for a short time with high concentrations and returned to sea water indicates a high capacity for recovery of this region. The higher activity of this region may also be demonstrated through differential conditioning. On continuous exposure to low concentrations larvae may develop exceedingly large oral lobes, although the solution is strong enough to inhibit oral and anal arms as well as to prevent the development of a concave ventral surface (Figs. 35, 36, 37, 42, 43).

The reaction of entoderm to pilocarpine is quite similar in many ways to that found with such agents as lithium and thiocyanate. Continuous exposure to high concentrations prevents the differentiation of entoderm or may cause the cells of the presumptive entoderm to become dissociated. In lower concentrations (M/500) invagination of entoderm takes place, but the cells of the invaginated gut may become dissociated with continuous exposure. Still lower concentrations permit gut to form and invagination to take place. A 12-hour exposure to high concentrations following fertilization seems to have a greater effect on ectoderm than on entoderm. A high percentage of exogastrulae is produced, however, by 24 hours' exposure (Fig. 23). These exogastrulae differ from those produced by some other inhibitory agents in that the ectoderm ordinarily undergoes little recovery. Long-time (48-hour) exposure to high concentrations followed by return to sea water produces a number of exogastrulae with a definitely enlarged gut and a diminished ectoderm (Fig. 32). Such entodermized forms, when produced by lithium, have been interpreted by Child to mean that the basal regions of prospective ectoderm have been inhibited to the physiological level of entoderm of early stages and in consequence of decreased apical dominance undergo the activation characteristic of entoderm as gastrulation approaches.

SUMMARY

1. Continuous exposure of fertilized sand-dollar eggs to M/100 pilocarpine retards cleavage and permits the development of only radial elongate bags of cells showing ciliary movement and a progressive dissociation into separate cells from the vegetal region.

2. Exposure of fertilized eggs for 12 hours to M/100 pilocarpine, followed by return to sea water, permits the development of plutei with anal arms at widened angles, asymmetrical, askew, or absent. Many distinctly inhibited, cell-filled larvae also appear with this treatment. Among the most interesting of these are some with a ciliated apical knob (rudimentary oral lobe), which represents a degree of differential recovery.

3. Exposures to M/100 pilocarpine for 24–48 hours, followed by return to sea water, tend to prevent the development of bilaterality, to inhibit gastrulation, and to produce a large number of cell-filled exogastrulae.

4. A solution of M/500 pilocarpine has a slight stimulating action on early cleavage, but after 11–12 hours' exposure distinctly inhibitory effects are noted. Larvae gastrulate in this concentration, but continued exposure produces a dissociation into separate cells of the invaginated entoderm. Development of ventrodorsality, oral lobe, and anal arms is also inhibited by continuous exposure. Removal of the developing larvae to sea water at certain intervals results in forms showing varying degrees of differential inhibition and recovery.

5. Continuous exposure to M/1,000 results chiefly in swollen, bilateral forms with an excess of internal cells, no anal or oral arms, and only a trace of skeleton. Continuous exposure to M/2,500 gives chiefly swollen, bilateral forms with widened oral lobes and shortened anal arms. The most noticeable effect of M/5,000 is an enlarged oral lobe. The enlarged oral lobes probably indicate differential conditioning.

6. Exposure of middle blastulae for 12½ hours to M/100 pilocarpine followed by return to normal sea water results in many aberrant larvae. The oral lobe is usually not developed, although in some cases it is considerably widened. Anal arms show many aberrancies. Most larvae have an excess of internal cells and many have excess skeleton. Most are bilateral, but some have retained their earlier radial symmetry.

7. Continuous exposure to M/1,000, beginning with the late blastula stage, results in larvae which usually show bilaterality, an excess of internal cells, a swollen appearance, and an enlarged oral lobe.

8. The recognition of differential inhibition, recovery, and conditioning resulting from pilocarpine treatment has aided in the discernment of certain critical periods and controlling factors in following the progressive development of a dynamic pattern in the larval sand dollar.

LITERATURE CITED

CHILD, C. M. 1936*a*. Differential reduction of vital dyes in the early development of echinoderms. Roux Arch. f. Entw'mech., **135**:426.

———. 1936*b*. A contribution to the physiology of exogastrulation in echinoderms. *Ibid.*, **135**:457.

———. 1940. Lithium and echinoderm exogastrulation: With a review of the physiological-gradient concept. Physiol. Zoöl., **13**:4.

RULON, O. 1938. Modification of development in the sand dollar with NaCNS and Ca-free sea water. Anat. Rec., Suppl., **72**, No. 4.

———. 1941. Modification of development in the sand dollar by NaCNS and Ca-free sea water. Physiol. Zoöl., **14**:305.

BUFFERING CAPACITY AND COMPOSITION OF THE BLOOD SERUM AND REGURGITATED DIGESTIVE JUICES OF THE MORMON CRICKET (ANABRUS SIMPLEX HALD.)[1]

(Two figures)

J. H. PEPPER,[2] F. T. DONALDSON,[3] AND ELLSWORTH HASTINGS[2]
Montana Agricultural Experiment Station, Bozeman, Montana

A SEARCH for more effective means of controlling outbreaks of the Mormon cricket, *Anabrus simplex* Hald., has disclosed, among other things, a lack of fundamental knowledge regarding the physiology of these insects. Recent studies have shown that the composition of insect body fluids may play an important role in the toxicity of insecticides. The investigation described in this paper was initiated to study the properties of the blood and regurgitated digestive juice of the Mormon cricket.

MATERIAL AND METHODS

The blood used in this study was obtained from adult crickets by puncturing the dorsal intersegmental membrane and collecting the exuded drops in a capillary tube. From 0.05 to 0.10 ml. of blood was obtained from each cricket, and approximately 6 ml., thus collected, was immediately centrifuged to remove the blood clots. Five-ml. portions of this serum were used for the determination of pH and buffering capacity, and in addition approximately 25 ml. of whole blood was collected for chemical analysis.

No special precautions were taken to prevent the possible loss of CO_2 from the sample to be measured, since Wigglesworth (1934, 1939) points out that there seems to be no chemical provision for its transportation in the insect blood and that CO_2 probably is eliminated from the insect body solely by the process of diffusion.

Bishop's data (1923) show that relatively large changes in the CO_2 tension of the honeybee body fluid are necessary to alter appreciably the pH values. It was found during this investigation that the pH of Mormon cricket blood remained constant for as long as 5 hours when held to 3°–4° C. after withdrawal, thus indicating that there was little change in the CO_2 equilibrium. When pH determinations were made on the blood of *Orthoptera* other than the Mormon cricket with methods described in this paper, values practically identical with those given by Bodine (1926) and others, for the same species, were obtained.

The crickets were starved for 24 hours to insure the absence of food material in the regurgitated digestive juice. Since they readily regurgitate their crop contents when handled, the juice was collected by holding a small vial under the mouth parts. From 0.05 to 0.10 ml. was obtained from each individual. For the determination of pH and buffering capacity approximately 6 ml. of juice was collected and centrifuged in order to remove any solids which might be present. Thirty ml. was collected for chemical analysis, this sample likewise being centrifuged to remove the solids present which inter-

[1] Contribution from the Montana State College, Agricultural Experiment Station, Paper No. 127 ("Journal Series").

[2] From the department of entomology. [3] From the department of chemistry.

fered with the withdrawal of uniform aliquots. After centrifuging, both the blood and the regurgitated digestive juices were kept at 2° C. until analyses were completed.

Preliminary trials of several methods for measuring pH and obtaining titration curves resulted in the selection of the glass electrode. The Cameron pH meter was used to obtain all the pH determinations reported in this paper.

The relatively high concentration of protein and the presence of appreciable color in the two fluids ruled out the colorimetric procedure as an accurate method. The main difficulty encountered with the bubbling hydrogen electrode was the production of excessive foam, necessitating the frequent addition of a foam-breaker. The quinhydrone electrode is unsatisfactory for measuring pH values above 7.5 but proved useful, as did the hydrogen electrode, for checking the normal pH value of the two fluids against the glass electrode.

Most of the methods employed in the chemical analyses of the two fluids were based on the procedures used in the clinical analysis of mammalian blood, as described by Peters and Van Slyke (1932). Since the digestive juice contained small amounts of silica, the reducing agent of Fiske and Subbarow (1925) was used in the colorimetric determination of phosphorus. Under the conditions employed, this reducing agent—1, 2, 4-aminonaphtholsulfonic acid—does not produce a blue color with silicomolybdic acid.

EXPERIMENTAL DATA

CHEMICAL ANALYSIS

The chemical analyses of the regurgitated digestive juice and the blood serum are shown in Table 1.

The color of the regurgitated digestive juice when first withdrawn from the cricket varied from dark brown to black, and after exposure to the air it turned a uniform black. It contained 19 per cent total solids. Proteins and nonprotein nitrogenous compounds were present in approximately equal amounts and together make up over half the total solids. The relatively high percentage of nonprotein nitrogen may have resulted from the 24-hour starvation period to which the crickets were subjected. Phosphorus was present chiefly in the inorganic form, although organic phosphorus accounted for 6.3 per cent of the total. Hoskins and Harrison (1934) in a qualitative examination of the ventricular contents of the honeybee failed to detect any organic phosphorus. Expressed in milligram equivalents, the potassium content of the juice was approximately twenty-five times the sodium content, while in the blood the sodium content exceeded that of the potassium.

Analyses were made on the blood serum rather than on the whole blood, for clotting took place soon after the blood was drawn. The addition of materials commonly employed to prevent clotting in mammalian blood had no effect on that of the cricket. The amount of clotted material formed in the cricket blood was very much smaller than that formed in mammalian blood. Only 1.8 per cent of the total nitrogen in the blood was removed with the clot. Immediately after withdrawal the blood was a clear straw color, but on exposure to the air it gradually changed to a dark brown. According to Wigglesworth (1939), this darkening is due to the formation of melanin.

Wigglesworth (1934) has pointed out that insect blood is characterized by high concentrations of amino acids and phosphates, and the data presented in this paper show that this is true in the case of cricket blood. This fluid had about the same concentration of amino acids and a much higher concentration of phosphate than Bishop *et al.* (1925)

found in the blood of honeybee larvae. The amount of total nitrogen in cricket blood was approximately the same as in honeybee larvae, but the proportion of nonprotein nitrogen, other than amino nitrogen, was much higher in the cricket blood. Magnesium and calcium in the cricket blood were much less abundant than in that of bee larvae. From this comparison it is apparent that not only are there large differences between mammalian and insect blood but also between the blood of different insects.

TABLE 1

CHEMICAL COMPOSITION OF THE BLOOD SERUM AND REGURGITATED DIGESTIVE JUICE OF THE MORMON CRICKET, *Anabrus simplex* HALD.

	MILLIEQUIVALENTS PER LITER	
	Blood Serum	Digestive Juice
Na	21.9	3.0
K	15.3	74.6
Ca	3.0	38.4
Mg	1.4	23.2
PO_4 (total)	97.4	104.0
PO_4 (inorganic)	58.9	97.4
N (total)	930.0	1279.0
N (protein)	495.0	606.0
N (nonprotein)	434.0	672.0
N (amino)	187.0	517.0
N (nonprotein other than amino)	247.0	155.0
Cl	.....	91.0
H* (calculated)	25.9	64.2
Total cations	67.5	203.4
Total anions	58.9	188.4
Total solids (gm. per liter)	94.8	197.5
Ash (gm. per liter)	5.68	11.0

* The figures given for H represent the H combined with the phosphate salts present in the two fluids at their normal pH values.

In both the digestive juice and the blood serum the total anions expressed as milliequivalents per liter are less than the total cations determined. However, chlorides, which were not determined in the blood, would add to the sum of the anions. It is probable that the two totals would not be equal in any case, for undoubtedly some of the cations were associated with organic anions such as amino acids and complex phosphorus compounds which were not determined. In calculating the total cations, account was taken of the hydrogen ions combined with phosphates at the normal pH values of two fluids.

pH VALUES AND BUFFER INDICES

The pH of the regurgitated digestive juice was 5.17. Hoskins and Harrison (1934) report pH values ranging from 4.6 to 6.3 for various parts of the digestive tract of the honeybee. Wigglesworth (1934) states that the gut contents of insects are slightly acid and that in general they tend to be more alkaline in phytophagous, and more acid in

carnivorous, insects. The cricket is usually a phytophagous insect, but under some conditions it may become strongly carnivorous. The 24-hour starvation period prior to collecting the juice may have contributed to its strongly acid character.

The titration curve for the regurgitated digestive juice is shown in Figure 1. The points on the curve represent averages of five determinations. The greatest variation in individual values for any point on the curve was 0.20 pH. The values for most of the points varied less than 0.10 pH.

The buffering-capacity curve shown in Figure 2 was obtained by plotting pH against β values.[4] The values of β were determined by Van Slyke's (1922) procedure of drawing

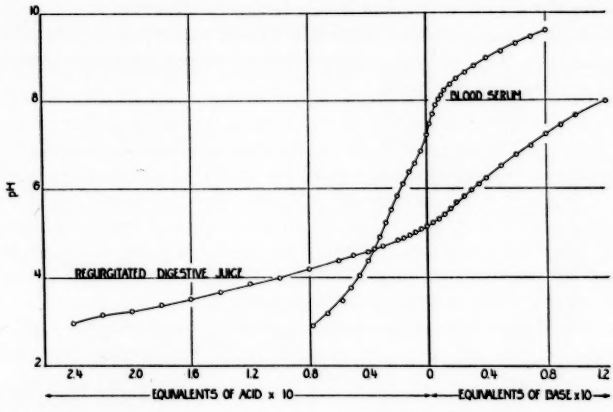


FIG. 1.—Titration curves for blood serum and regurgitated digestive juices of the Mormon cricket (*Anabrus simplex* Hald.).

tangents to the titration curve. Small deviations from a smooth titration curve can easily result in large variations in β values when the tangent method is used. For this reason maximum points on the buffering-capacity curve should be viewed with skepticism, especially where biological systems are being investigated. In such systems conclusions drawn from pH measurements should be based on several replications, as exact measurements are difficult to obtain.

No distinct inflection point occurred at pH 5.17—the normal pH of the digestive juice—or at any other point throughout the range investigated. At a pH of 5.17 the ratio of dibasic to monobasic phosphate is given by the equation

$$5.17 = PK' + \log \frac{B_2HPO_4}{BH_2PO_4}.$$

[4] β being defined as follows:

$$\beta = \frac{dB}{dpH} \quad \text{or} \quad \frac{dA}{dpH},$$

where B and A are equivalents of base and acid per liter, respectively.

Taking the value of PK' as 6.9 (Cohn, 1927) and solving for the ratio B_2HPO_4/BH_2PO_4, it is found that at a pH of 5.17, over 95 per cent of the phosphate is present as the monobasic salt. Under these conditions the phosphate system has practically no buffering action, and one would not expect it to produce any maximum β value at this point. On the basis of the data available it is impossible to designate any individual buffering system, but this does not imply that such systems do not occur.

Wigglesworth (1934) states that the blood reaction in insects is usually very slightly acid and also that it may vary with sex and age. The pH of the Mormon cricket blood serum was found to be 7.23, which is somewhat higher than Bodine (1926) found for a number of other *Orthoptera*. The highest value that he reported for any insect investigated was 7.04 for the blood of the last instar larvae of *Pieris rapae*.

In the titration curve for the blood, shown in Figure 1, the points plotted are averages for five determinations. The variations in individual values were considerably less than those recorded for the digestive juice.

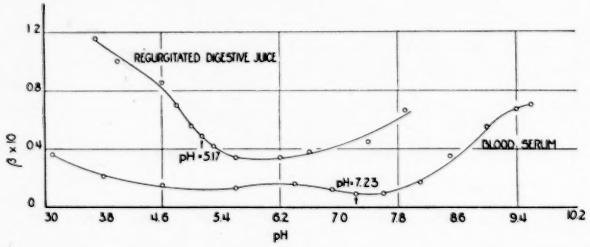


FIG. 2.—Buffering-capacity curves for the blood serum and regurgitated digestive juices of the Mormon cricket (*Anabrus simplex* Hald.).

It will be noted that smooth titration curves containing no clear-cut inflection points were obtained for both the blood and the digestive juice. No doubt this is due to the presence of a great many buffering systems and the consequent overlapping of their buffering effects.

Both fluids contain a high percentage of amino acids. The buffering effects of these amino acids would be spread over a wide range due to their varying PKa values. In this respect insect blood is not comparable to mammalian blood which has a low amino acid concentration.

The curve for the buffering capacity of the blood (Fig. 2) was obtained in the same manner as that for the digestive juice. It will be noted that the blood has little buffering capacity at its normal pH (7. 23).

There is a slight increase in the β values on the acid side of 7.23. These values become slightly lower in the neighborhood of pH 5 and then rise slowly. On the alkaline side the β values increase more rapidly. In other words, the blood has a greater buffering effect for bases than for acids. It may readily be shown that the greater buffering effect on the alkaline side cannot be due to the phosphate system. At a pH of 7.23 the ratio of dibasic to monobasic phosphate is 2.14 to 1, or 68 per cent of the phosphate is present as the dibasic salt. As the pH is increased above 7.23, the proportion of dibasic salt will become greater. At the same time the buffering capacity of the phosphate system will decrease

and reach a minimum at a pH of approximately 9.5. The uniformity of the β values for the blood in the vicinity of its normal pH indicates that no single buffering system is responsible for the buffering capacity. When the buffering effects overlap to give uniform β values, it becomes difficult to differentiate between inflections on the titration curve due to a single buffer system and inflections due to slight errors of measurement. For example, in the case under discussion shifting the titration curve only 0.10 pH in the vicinity of 6.9 could produce a maximum in the buffering-capacity curve corresponding with the effect expected from a phosphate system. Consequently, it is only safe to conclude that the buffering capacity at the normal pH of the blood is probably due to a combination of buffering systems.

SUMMARY

A study of the regurgitated digestive juice and the blood serum of the Mormon cricket, *A. simplex* Hald., revealed the following facts:

1. The regurgitated digestive juice contained high concentrations of amino acids, phosphates, and potassium and a very low sodium concentration.

2. A buffering-capacity curve derived from a titration curve indicated that the juice did not show a maximum buffering effect at its normal pH of 5.17.

3. Sufficient data were not available to designate a buffering system at this point, but it was shown that the phosphate system could not exert much effect.

4. The blood serum likewise contained a high concentration of amino acids and phosphates. Expressed in milliequivalents the ratio of sodium to potassium was 1.4:1 compared with a ratio of 1:25 in the regurgitated digestive juice. The total and amino nitrogen content of the cricket blood serum was similar to that reported for the blood of honeybee larvae, but the inorganic constituents differed considerably.

5. The buffering-capacity curve showed that at the normal pH, 7.23, the blood had little buffering effect for acids and only slightly more for bases. Amino acids, which are not important buffering agents in mammalian blood, are probably responsible for a large part of the buffering action in cricket blood, due to their high concentrations.

LITERATURE CITED

BISHOP, G. H. 1923. Body fluid of the honeybee larvae. Jour. Biol. Chem., **58**:543–565.

BISHOP, G. H., BRIGGS, A. P., and RONZONI, E. 1925. Body fluids of the honeybee larvae. Jour. Biol. Chem., **66**:77–88.

BODINE, J. H. 1926. Hydrogen ion concentration in the blood of certain insects (*Orthoptera*). Biol. Bull., **51**:363–369.

COHN, E. J. 1927. The activity coefficient of the ions in certain phosphate solutions. Jour. Amer. Chem. Soc., **49**:173.

FISKE, G. H., and SUBBAROW, Y. 1925. The colorimetric determination of phosphorus. Jour.Biol.Chem., **66**:375–400.

HOSKINS, W. M., and HARRISON, A. S. 1934. The buffering power of the contents of the honeybee and its effect upon the toxicity of arsenic. Jour. Econ. Ent., **27**:924–942.

PETERS, J. P., and VAN SLYKE, D. D. 1932. Quantitative clinical chemistry, Vol. **2**. Baltimore: Williams & Wilkins Co.

VAN SLYKE, D. D. 1922. On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. Jour. Biol. Chem., **52**:525–570.

WIGGLESWORTH, V. B. 1934. Insect physiology. London: Methuen & Co., Ltd.

———. 1939. The principles of insect physiology. New York: E. P. Dutton & Co., Inc.

SOME BLOOD CONSTITUENTS OF THE NORMAL SKATE[1]

(Eleven figures)

F. A. HARTMAN, L. A. LEWIS, K. A. BROWNELL
F. F. SHELDEN, AND R. F. WALTHER
Marine Biological Laboratory, Woods Hole, Massachusetts, and the Department of Physiology, Ohio State University, Columbus

IN PREPARATION for a study of the chemical changes produced by interrenalectomy in the skate we needed to know the values for normal animals. Smith (1929) has published normal values for electrolytes and urea in *Raja laevis* and *R. diaphenes*. At Woods Hole the most satisfactory animal for our purpose was the common skate, *R. erinacea*, because it was plentiful and it could be interrenalectomized without too much difficulty. Little or no data have been published on the electrolytes and urea in this species. Moreover, too little attention to the effect of inanition has been given. Kisch (1930) has shown that starvation causes a fall in blood urea, but he did not determine the electrolytes.

We studied the range of variation in a large number of animals in both blood and perivisceral fluid. Then the effects of inanition and feeding were followed after it was found that plasma sodium rose with the fall in blood urea. Blood sugar and volumes per cent of erythrocytes were also determined.

METHODS

All animals reported in this paper belonged to the species *R. erinacea*. They were collected by dredging, in regular fish traps, or occasionally by hook and line. Dredging possesses the advantage that the animals are likely to be in a good state of nutrition when caught. However, skates, because of their structure, are prone to injury by pressure. Therefore to avoid this in dredging it is necessary to drag slowly and empty the net frequently.

The skates were usually kept either in a large lead-lined tank or in small, shallow wooden tanks. A few were kept in glass or concrete aquaria. All were supplied with running sea water. The water for the lead-lined tank was maintained at 10°–12° C., while the temperature of the water for the other tanks was 16°–20° C.

Feeding.—It is possible to induce some skates to eat in an aquarium after they have had 3 or 4 weeks to become accustomed to their surroundings. Neither time nor space permitted us to condition animals in this manner. We resorted to forced feeding. A glass tube about 12 mm. in inside diameter and 2 feet long was used. The desired quantity of small live *Fundulus* or ground squid or clam was forced into the tube by means of a wooden rod. Then the tube was inserted as far as the bottom of the stomach with as little struggling of the fish as possible. The contents were ejected from the tube with the wooden plunger. In this manner from 3 to 6 *Fundulus* or 15–20 cc. of ground squid or clam were introduced at one time. The animals were fed every 3 or 4 days. Sometimes

[1] Aided by grants from the Committee on Research in Endocrinology of the National Research Council and the Ohio State University Comly Fund.

the food was regurgitated within a few hours. If this was discovered, feeding was repeated.

Blood was drawn either from the heart or from the tail by gentle suction through a 20-gauge needle. The animal was fastened, back down, on a padded concave board. Running water entered one spiracle through a rubber tube. The quantity of blood

TABLE 1

PLASMA ELECTROLYTES IN NORMAL SKATES (*Raja erinacea*)

(Samples obtained within 24 hours after removal from the sea)

No.	Weight (Gm.)	Sodium (mEq./l.)	Potassium (mEq./l.)	Calcium (mEq./l.)	Magnesium (mEq./l.)	Chloride (mEq./l.)	Urea (mM/l.)	Erythrocytes (Vol. Per Cent)	Glucose (Mgm. Per Cent)
Male:									
44	3,400	241	5.7	11.4	6.1	252	275	18.5	58
62	3,700	244	11.9	10.6	2.0	263	343	22.0	.....
65	3,800	254	11.7	9.8	1.4	267	347	22.0	73
66	3,150	260	10.0	11.2	2.8	265	322	23.0	61
68	2,625	245	6.0	12.4	.....	248	335	20.0	52
87	1,975	266	6.0	13.4	1.8	268	328	20.0	62
88	2,100	246	8.9	11.2	5.0	270	308	18.0	58
Female:									
43	2,050	231	8.1	11.8	4.2	250	355	22.0	37
46	2,425	244	7.9	11.9	5.0	246	380	20.0	34
50	3,100	262	7.8	13.6	5.6	259	302	17.0	65
60	3,875	266	4.8	11.3	3.0	236	342	18.5	.....
61	3,900	240	6.1	11.2	2.7	240	312	17.5	.....
63	1,475	265	8.1	12.3	2.0	258	342	21.0	57
64	1,350	243	10.1	11.2	4.0	261	305	24.0	71
67	3,100	230	8.2	12.0	2.7	245	350	18.0	65
69	3,100	246	7.3	12.0	.....	279	332	15.0	38
70	2,825	287	7.8	.....	.....	263	332	20.0	55
71	2,450	272	5.7	12.4	.....	253	343	18.0	53
85	1,600	256	6.4	11.8	6.2	254	316	18.0	38
86	3,800	263	8.9	13.8	5.9	258	303	20.0	63
89	3,850	256	11.0	14.2	3.9	246	321	19.0	75
90	2,425	252	6.0	13.0	3.5	253	323	17.0	50
92	1,700	230	11.2	.....	.....	250	327	.....	50
94	3,275	258	7.9	.....	.....	277	315	15.5	46
97	1,400	237	6.9	.....	.....	254	328	20.0	33
98	1,525	251	7.6	.....	.....	263	327	17.5	28
99	1,800	241	8.9	.....	.....	240	328	19.5	55

drawn at one time was most often 2.5–3.0 cc., occasionally 5 cc. Blood sugar was determined on this blood, which was collected without anesthetic and might on this account be affected by stimulation.

Chemical determinations were made according to the following methods: sodium, Butler and Tuthill (1931); potassium, Consolazio and Talbott (1938); calcium, Halverson and Bergeim (1917); magnesium, Briggs (1922); chloride, Peters and Van Slyke (1932); urea, Van Slyke aeration method; glucose, Somogyi's modification of the Shaffer-Hartmann method (1937); erythrocyte volume per cent, Wintrobe (1933).

RESULTS

The samples on which these determinations were made were taken from animals which were in good condition as indicated by appearance and activity. Some were recently caught while others had been kept for variable periods. More than 100 animals were used. The animals varied greatly in size as shown in Tables 1 and 2.

The different electrolytes for the same animal are shown in Table 1. No close correlation can be found between these values. The sodium appeared to vary independently of the chloride or of the urea. Despite this, it will be shown later that plasma sodium varies inversely with the blood urea in the same individual.

TABLE 2

ELECTROLYTES IN NORMAL SKATES

No.	Weight (Gm.)	Sodium (mEq./l.)		Potassium (mEq./l.)		Calcium (mEq./l.)		Magnesium (mEq./l.)		Chloride (mEq./l.)		Urea (mM/l.)	
Male:													
40.....	3,550	*241**	236†	*9.3*	8.1	*10.6*	6.7	*3.9*	42.0	*255*	303	*340*	374
65.....	3,800	*254*	234	*11.7*	13.4	*9.8*	5.8	*1.4*	18.9	*267*	300	*347*	349
66.....	3,150	*260*	264	*10.0*	12.7	*11.2*	6.6	*2.8*	19.8	*265*	298	*322*	367
76.....	3,800	*288*	264	*9.5*	12.5	*13.8*	4.6	*2.5*	30.0	*277*	262	*254*	325
79.....	2,575	*267*	254	*6.6*	9.0	*11.2*	8.6	*4.3*	27.0	*250*	239	*297*	330
87.....	1,975	*266*	318	*6.0*	11.8	*13.4*	7.8	*1.8*	19.6	*268*	260	*328*	353
88.....	2,100	*246*	246	*8.9*	13.0	*11.2*	7.2	*5.0*	22.5	*270*	272	*317*	346
Female:													
67.....	3,100	*230*	202	*8.2*	10.9	*12.0*	12.2	*2.7*	28.7	*249*	308	*349*	294
73.....	2,500	*244*	240	.....	6.7	*13.4*	11.4	*5.2*	27.0	*253*	276	*334*	367
77.....	3,500	*259*	245	*7.3*	8.2	*12.4*	7.4	*6.5*	28.0	*259*	306	*337*	327
78.....	1,650	*249*	230	*6.3*	14.2	*12.4*	7.0	*2.8*	25.0	*258*	283	*322*	349
80.....	1,575	*251*	250	*4.3*	8.1	*11.8*	10.5	*3.8*	23.0	*238*	303	*352*	330
86.....	3,800	*263*	201	*8.9*	11.9	*13.8*	17.4	*5.9*	25.0	*258*	297	*303*	349
89.....	3,850	*256*	219	*11.1*	8.7	*14.2*	17.2	*3.9*	14.2	*246*	323	*321*	371
90.....	2,425	*252*	231	*6.0*	9.9	*13.0*	10.7	*3.5*	22.0	*253*	239	*323*	348

* Italic figures indicate plasma and blood urea.

† Perivisceral fluid.

A comparison of the plasma with the perivisceral fluid was made in 41 individuals. No remarkable difference was shown in the sodium values in the two fluids. Although the 15 animals chosen at random from this group for Table 2 happened to show some difference, this was not so for the whole group.

Potassium was more concentrated in the perivisceral fluid in 32 out of 37 cases. Calcium was less concentrated in the perivisceral fluid in 16 out of 22 cases. Magnesium was much more concentrated in the perivisceral fluid in all cases. Chloride was greater in the perivisceral samples in 34 out of 41 cases. Urea was greater in the perivisceral fluid in 20 out of 28 cases. Thus, in general, it may be said that magnesium, potassium, chloride, and urea were more concentrated in the perivisceral fluid, while calcium was less concentrated.

It should be noted that the perivisceral fluid varied greatly in volume. Sometimes there was too little for analysis; then again the cavity might be so gorged that the fluid welled out on puncture. No relation was observed between the volume of the fluid and

the concentration of urea and electrolytes. A comparison of the values for each substance was made in the blood or plasma of many different individuals.

Sodium.—The distribution of plasma sodium is shown in Figure 1. The extremes were 219 and 289 mEq./l. The mean value was 254 mEq./l. Two-thirds of the values lay between 240 and 270 mEq./l. The range shown in samples taken from fish within 24 hours after removal from the sea is practically the same as that for fish kept over a longer period.

Sodium distribution in the perivisceral fluid shows a much wider range. The lowest values were 201 and 202, and the highest were 304, 318, and 463 mEq./l. These are not shown in the figure.

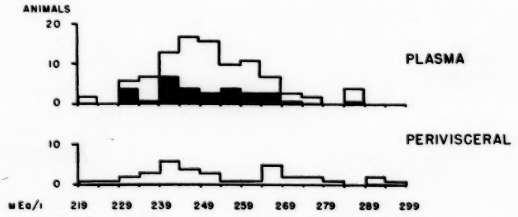


FIG. 1.—Distribution of sodium in the skate. Those tested within 24 hours of removal from sea shown in black.

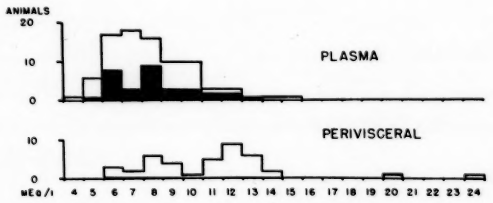


FIG. 2.—Distribution of potassium in the skate. Those tested within 24 hours of removal from sea shown in black.

Potassium.—Hemolysis occurs rather easily in skate's blood, making the potassium values high. Whenever there was evidence of hemolysis the potassium determinations were discarded.

Plasma potassium ranged from 4 to 15 mEq./l., with the mean at 8. The range of the values in animals sampled within 24 hours after removal from the sea was not quite so great. In perivisceral fluid, potassium ranged from 6 to 24 mEq./l. with the mean at 10. Thus there was a tendency for potassium to be more concentrated in the perivisceral fluid (Fig. 2).

Calcium.—Plasma calcium lay between 6 and 14 mEq./l., with a mean of 12. The perivisceral calcium ranged from 3 to 18 mEq./l. (Fig. 3). Generally speaking, it was less concentrated than in the plasma.

Magnesium.—Plasma magnesium ranged between 2 and 7 mEq./1., with a mean of

5 (Fig. 4). The perivisceral magnesium concentration varied so greatly that it could not be charted conveniently. It extended from 6 to 98 with a mean of 42 mEq./l.

Chlorides.—The plasma chlorides ranged from 230 to 285, while the mean was 255 mEq./l. (Fig. 5). Samples taken not more than 24 hours after capture of the fish gave a similar range. The range in the perivisceral fluid was greater, being from 235 to 330 for all except two samples not shown in the chart, which were 385 and 400 mEq./l. The mean was 300 mEq./l., which is a very definite shift toward greater concentration.

Urea.—The blood urea ranged from 254 to 384 mM/l., with a mean value of 320 (Fig. 6). The range was not so great in animals tested within 24 hours of removal from

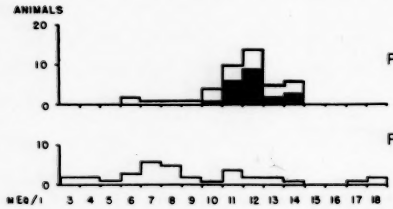


FIG. 3

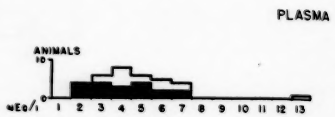


FIG. 4

FIG. 3.—Distribution of calcium in the skate. Those tested within 24 hours of removal from sea shown in black.

Fig. 4.—Distribution of magnesium in the skate. Those tested within 24 hours of removal from sea shown in black.

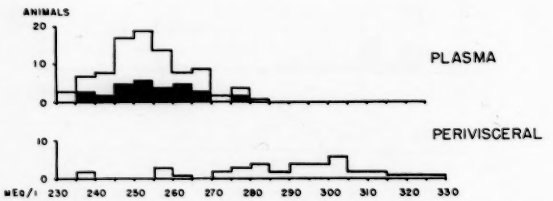


FIG. 5.—Distribution of chloride in the skate. Those tested within 24 hours of removal from sea shown in black.

the sea. The small series from perivisceral fluid showed a possible shift toward greater concentration.

Erythrocytes.—The volume per cent of erythrocytes ranged from 11 to 24, with the mean at 18. In animals sampled within 24 hours of removal from the sea the lowest value was 15 and the mean was 19 (Fig. 7).

Blood glucose.—The blood glucose ranged from 0 to 105 mgm. per cent, with a mean of 45. In fish tested within 24 hours of removal from the sea the range was 30–75 mgm., with a mean of 54 mgm. per cent (Fig. 8).

Recapitulating, our results indicate that skates recently removed from the sea have a smaller range of blood urea and blood sugar and a higher volume per cent of erythrocytes than do skates removed for a longer period. Noteworthy are the higher values of

potassium, urea, magnesium, and chloride, especially the last two, in the perivisceral fluid as compared to those in plasma.

The differences observed between tests made on skates within 24 hours after their removal from the sea and those made after a longer period led us to study the effects of inanition and feeding.

Effect of inanition.—The effect of inanition was studied in 10 skates. Six of them probably started the experiment as fed animals, because they were used within 24 hours after dredging them from the sea. Evidence that this was so was furnished by 7 skates killed immediately after removal from the sea. In all cases the alimentary canal contained food in some stage of digestion. Food was present in the stomach and spiral

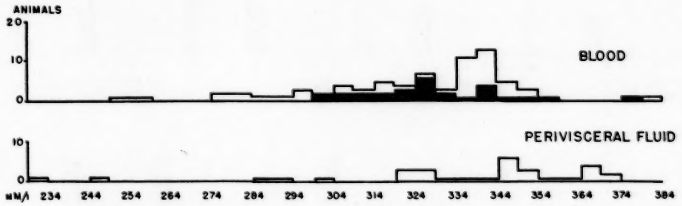


FIG. 6.—Distribution of urea in the skate. Those tested within 24 hours of removal from sea shown in black.

FIG. 7

FIG. 8

FIG. 7.—Distribution of erythrocyte volume per cent in the skate. Those tested within 24 hours of removal from sea shown in black.

FIG. 8.—Distribution of blood sugar in the skate. Those tested within 24 hours of removal from sea shown in black.

valve of all but 1, which contained food only in the spiral valve. Blood samples were drawn from the 6 animals (Nos. 45, 46, 51, 62, 123, and 44 [Fig. 9]) after 7 days' starvation. All showed considerable increase in plasma sodium. The blood urea was decreased in all but 1 (No. 44). This decrease was considerable in 4.

Four additional animals (Nos. 60, 61 122, and 124 [Fig. 9]), which had been kept for several days before the experiment was started, were observed for longer periods. They showed similar but more gradual changes, plasma sodium increasing and blood urea decreasing.

Plasma chloride was increased at the same time that the sodium rose. The values were as follows: **44,** 252, 278; **45,** —, 282; **46,** 246, 279; **51,** 270, 274; **60,** 236, 307; **61,** 240, 266, 278; **62,** 263, 284; **122,** 233, 236, 252, 258, 264, 314; **123,** 258, 260; **124,** 240, 250, 254, 260, 264, 326 mEq./l.

Plasma calcium was determined in 7 of the skates. It ranged from 11 to 13 mEq./l. and showed little or no change during inanition.

Plasma magnesium was determined in 5 skates. It showed an increase with inanition. The values were as follows: **44,** 6.1, 9.2; **51,** 4.9, 6.0; **60,** 3.0, 6.6; **61,** 2.7, 4.0, 4.3; **62,** 2.0, 2.3 mEq./l.

In sugar determinations there was considerable variation, due in part, no doubt, to the stimulation attendant upon obtaining the blood. However, later samples were often

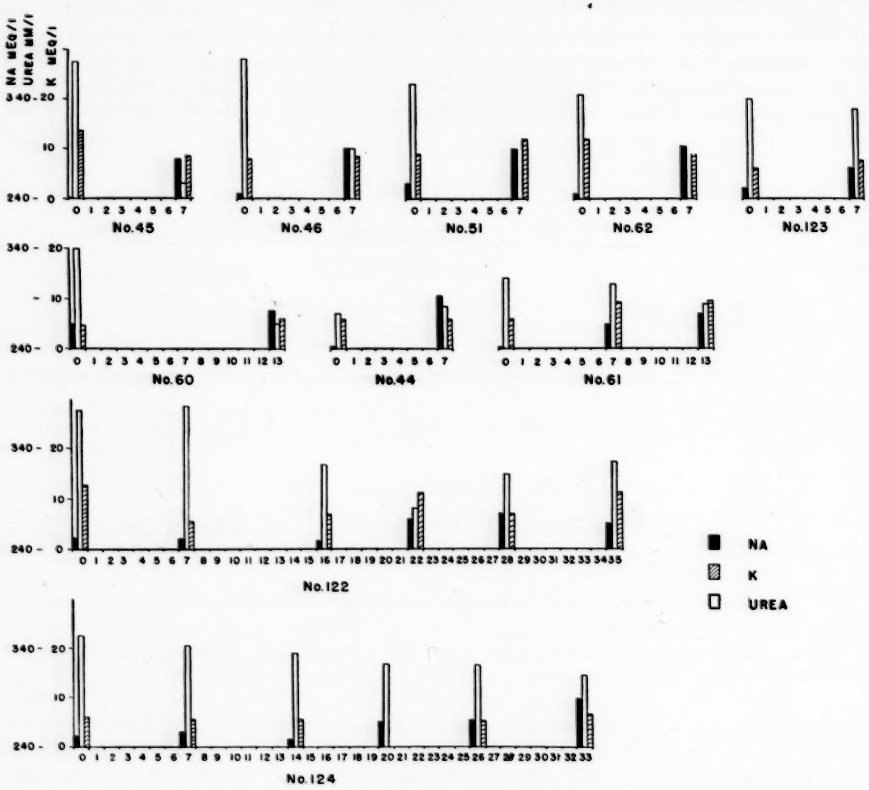


FIG. 9.—Blood changes during inanition

higher than early ones. The values corresponding to the same time intervals and in the same order as shown in Figure 9 for the electrolytes were as follows: **44,** 58, 75, 68; **45,** 43, 70; **46,** 34, 23; **60,** —, 18, 25; **61,** —, 25, 77; **122,** 43, 25, 50, 80, 112, —; **123,** 68, 67; **124,** 30, 27, 40, 64 mgm. per 100 cc.

The volume per cent of erythrocytes was also determined when other determinations were made, in all animals except Nos. 45 and 123. In only 1 animal was there more than a 12 per cent change, usually a fall. The values were as follows: **44,** 18.5, 16.5, 16.5; **46,** 20.0, 18.0; **60,** 18.5, 15.0, 13.0; **61,** 17.5, 18.0, 16.5; **122,** 17.0, 16.0, 17.5, 18.0, 18.0; **124,** 16.0, 16.0, —, 18.0 volume per cent.

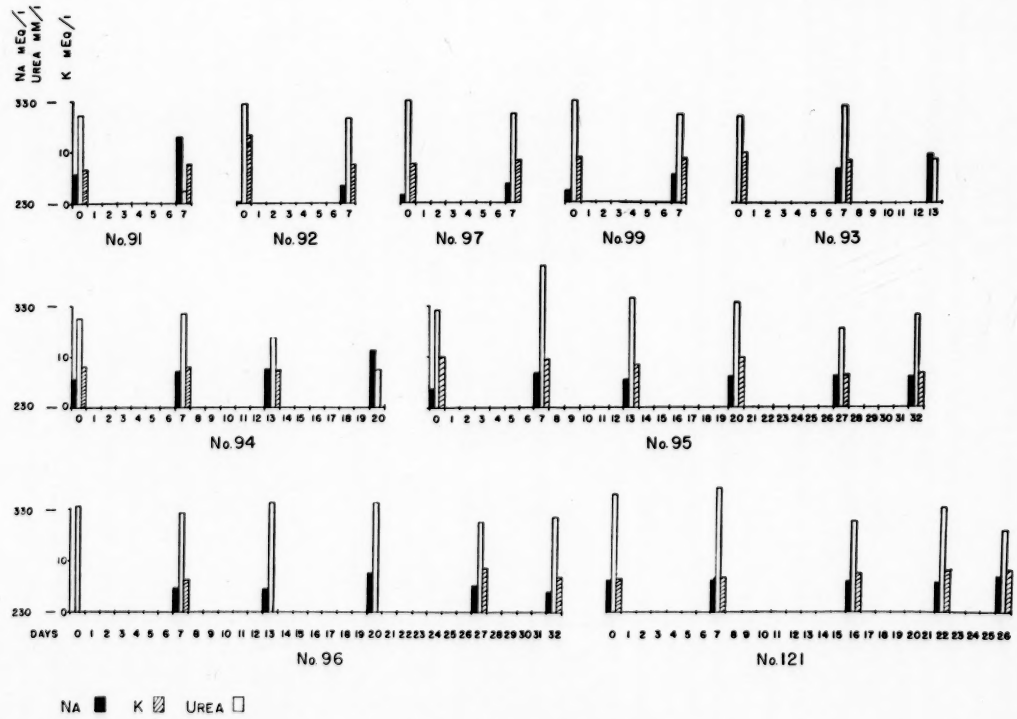


FIG. 10.—Blood changes in fed animals

It should be stated that only 2 of the animals (Nos. 122 and 124) were killed, the others dying 3–7 days after the last sample was taken.

Thus it is seen that the typical changes in inanition are a fall in blood urea and a rise in plasma sodium, chloride, and possibly magnesium. In order to prove that these changes were due to inanition, another series of skates were kept under similar conditions but fed as described earlier.

Effect of feeding.—Nine skates were fed for periods of time ranging from 7 to 32 days. Three of them showed a decided drop in blood urea (Fig. 10, Nos. 91, 93, and 94). The others showed a minor reduction. However, in general it could be said that these changes were not so great as in the unfed animals. The plasma sodium increased as in inanition but to a lesser degree.

Plasma chloride was more variable, increasing in some animals but showing little change in others. The values were as follows: **91,** 256, 307; **92,** 250, 276; **93,** —, 242, 260; **94,** 277, 268, 256, 286; **95,** 253, 254, 250, 261, 256, 256; **96,** 266, 257, 242, 273, 257, 251; **97,** 254, 230; **99,** 240, 263; **121,** 245, 248, 264, 249, 255 mEq./l.

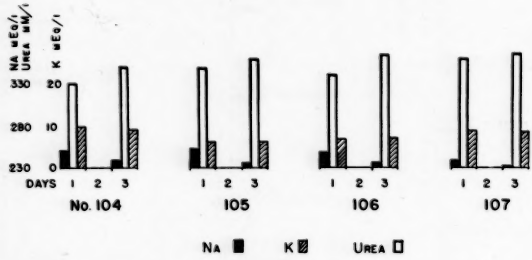


FIG. 11.—Effect of urea injection on plasma sodium

Blood sugar varied as in the starved animals. The values were as follows: **91,** 52, 68; **92,** 50, 38; **97,** 33, 55; **99,** 55, 54; **93,** 26, 33; **94,** 46, 103, 143, 177; **95,** 51, 37, 26, 18, 18, 25; **96,** 27, —, 28, 30, 28, 37; **121,** 56, 40, 31, 54, 55 mgm. per 100 cc.

The volume per cent of erythrocytes showed little change. The values in 6 skates were as follows: **91,** 18.0, 16.5; **92,** 15.0, 14.5; **97,** 20.0, 21.0; **99,** 19.5, 21.0; **93,** 16.0, 16.5, 16.5; **94,** 15.5, 14.5, 15.0, 16.0; **95,** 19.0, 17.0, 17.5, 16.5, 15.0, 15.0; **96,** 15.0, 15.0, 15.5, 14.0, 14.0, 14.5; **121,** 18.0, 14.0, 14.0, 15.0 vol. per cent.

Three of the animals were killed or used for other investigations. In spite of the feeding, the others died in from 3 to 7 days after the last determinations were made.

It can be seen from the above results that the fall in blood urea and rise in plasma sodium are in the same direction as in inanition but that they are less marked. Therefore, inanition appears to be a causative factor.

A fall in blood urea is accompanied by a rise in plasma sodium. If it could be shown that a rise in urea is accompanied by a fall in plasma sodium, the change in urea concentration would appear to be responsible for the changes in sodium concentration. Experiments were undertaken to demonstrate this point.

Effect of urea injection on plasma sodium.—The effect of blood urea concentration on plasma sodium was determined by the injection of urea into 4 normal skates, weighing

between 975 and 1,450 gm. They had been caught 6 days before beginning the experiments. The injections were made daily into the muscle of the tail. A 25 per cent solution of urea was employed. Two animals received 1 cc. per kilo body weight, and 2 received double this dose. The injections were continued for 3 days. Blood samples were taken just before the first injection and 24 hours after the last injection.

The blood urea was increased, as would be expected, while the plasma sodium was decreased. The double dose of urea produced no greater effect than the smaller dose. The significant point is that the plasma sodium fell with the rise in blood urea in all experiments. The results are shown in Figure 11.

DISCUSSION

The relatively short survival of many skates under laboratory conditions is difficult to explain. The various factors concerned might be lack of food, temperature, pressure, injury, infection, or intoxication.

Simpson and Ogden (1932) found that dogfish died if the temperature rose above 18° C. Although most of our animals were maintained at 10°–12° C., some that survived the longest were kept at 16°–20° C. Therefore, temperature was not a major factor. Undoubtedly, inanition was a factor in unfed animals, but many fed animals survived no longer than those unfed. However, the feeding was inadequate because all fed animals lost weight. More frequent feeding was indicated. Reduced pressure might have contributed to the short survival. Skates are usually found at depths of 3 meters or more. The water in our tanks was 0.6 meter deep or less. Change in pressure itself could scarcely be responsible, except that it may have contributed to the discontent shown by many animals. Occasionally, undetected injury might have occurred when the fish were caught. There was no reason to believe that the bacterial pollution of the water which supplied the aquaria was sufficient to be a factor, although sewage was present in the general region from which the water was pumped. We found no evidence that the type of aquarium was a factor in the survival. Frequent attempts to escape indicated that many of the skates were disturbed by the confinement.

It will be noted that some of our animals were used after they had been in the laboratory for several days. This did not seem to vitiate the experiment, because the results were comparable with those obtained from freshly caught skates.

The observation that sodium and urea concentrations in the plasma tend to reciprocate each other in the same individual supports the idea that urea plays a part in osmotic regulation (Smith, 1936). Although feeding retarded the fall in urea, it did not prevent it. This would indicate that the amount of food was inadequate.

Para (1936) studied the effect of successive bleedings on the blood of the skate. He bled as many as six times at intervals of a few days, removing 30 cc. each time. His animals weighed 4–6 kgm. We never removed more than 5 cc. at one time, usually 3 cc. or less. In other words, in proportion to the body weight he removed about five times as much blood as we did. He found that the serum chloride increased after several days. This increase must have been due to inanition as well as to loss of urea contained in shed blood.

The great range in blood glucose is very striking. The higher values might be caused by stimulation associated with sampling. It is interesting that such low blood sugar values were found in apparently normal animals.

We confirmed Smith (1929) in finding that the calcium of the perivisceral fluid was somewhat less than that in the plasma. We differ from him in finding that the concentration of potassium in perivisceral fluid was somewhat greater than that in the plasma. Perivisceral fluid chloride, likewise, was somewhat greater than that in plasma.

The difference in concentration between substances in the perivisceral fluid and the blood indicates that the blood vessels exercise a selective influence on exchanges with the perivisceral fluid. The greater concentration of potassium, urea, magnesium, and chloride in the perivisceral fluid would indicate their excretion from the blood via this route. The abdominal pores make it possible for the perivisceral fluid to escape from the body.

SUMMARY

Plasma electrolytes, urea and sugar in the blood, and electrolytes and urea in perivisceral fluid were determined in a large number of skates belonging to the species *R. erinacea*. The concentrations of potassium, magnesium, chloride, and urea were greater and that of calcium less in perivisceral fluid than in plasma. Skates recently removed from the sea showed a smaller range of blood urea and blood sugar and a higher volume per cent of erythrocytes than those removed for a longer period. During inanition blood urea fell while plasma sodium, magnesium, and chloride increased. The volume per cent of erythrocytes tended to decrease. Feeding retarded these changes. A rise in blood urea from injection of this substance caused a fall in plasma sodium. Thus concentrations of urea and sodium tend to be complementary.

We wish to thank Dr. C. M. Osborn of the Woods Hole Oceanographic Institute for dredging facilities and Mr. R. A. Goffin for the facilities of the United States Bureau of Fisheries.

LITERATURE CITED

BRIGGS, A. P. 1922. A colorimetric method for the determination of small amounts of magnesium. Jour. Biol. Chem., **52**:349–355.

BUTLER, A. M., and TUTHILL, E. J. 1931. An application of the uranyl zinc acetate method for determination of sodium in biological material. Jour. Biol. Chem., **93**:171–180.

CONSOLAZIO, W. V., and TALBOTT, J. H. 1938. Modification of the method of Shohl and Bennett for the determination of potassium in serum and urine. Jour. Biol. Chem., **126**:55–61.

HALVERSON, J. O., and BERGEIM, O. 1917. The determination of small amounts of calcium, particularly in blood. Jour. Biol. Chem., **32**:159–170.

KISCH, B. 1930. Harnstoffuntersuchungen bei Selachiern. Biochem. Zeitschr., **225**:197–207.

PARA, E. 1936. L'Influence des saignées successives sur la composition chimique et physico-chimique du sang des animaux marins. Jour. physiol. path. gén., **34**:735.

PETERS, J. P., and VAN SLYKE, D. D. 1932. Quantitative clinical chemistry, **2**:834. Baltimore: Williams & Wilkins.

SIMPSON, W. W., and OGDEN, E. 1932. The physiological significance of urea. I. The elasmobranch heart. Jour. Exper. Biol., **9**:1–5.

SMITH, H. W. 1929. The composition of the body fluids of elasmobranchs. Jour. Biol. Chem., **81**: 407–419.

SMITH, H. W. 1936. The retention and physiological role of urea in the elasmobranchii. Biol. Rev., **11**: 4–82.

SOMOGYI, M. 1937. A reagent for the copper-iodimetric determination of very small amounts of sugar. Jour. Biol. Chem., **117**:771–776.

WINTROBE, M. M. 1933. Macroscopic examination of the blood. Amer. Jour. Med. Sci., **185**:58.

INDEX TO VOLUME XIV

Alteration of developmental pattern in the sand dollar by pilocarpine, The, 461

Ashkenaz, E. W., 281

Bissonnette, Thomas Hume, 379

Bodenheimer, F. S., 186

Brownell, K. A., 476

Buffering capacity and composition of the blood serum and regurgitated digestive juices of the Mormon cricket (*Anabrus simplex* Hald), 470

Charipper, A., 366

Child, C. M., 449

Constituents of the normal skate, Some, 476

D'Angelo, Savino A., 366

Danielson, Ragnar N., 96

Davenport, Demorest, 178

Differential modifications of coelom development in the starfish, *Patiria miniata*, 449

Donaldson, F. T., 470

Eastlick, Herbert L., 136

Edelmann, Abraham, 373

Effect of cyanide on oxygen consumption of *Colpidium campylum*, The, 193

Effect of thymus gland, thymus extracts, and thymus derivatives on growth and metamorphosis of amphibian larvae, 366

Effects of acetylcholine, atropine, and nicotine on the isolated heart of the commercial crab, *Cancer magister* Dana, The, 178

Effects of modified Ringer solutions on head reconstitution in *Dugesia* (=*Euplanaria*) *dorotocephala*, 316

Effects of peripheral inhibition on the muscle action potentials of the crab, The, 296

Evans, F. R., 227

Evans, Titus Carr, 163

Experimental modification of breeding cycles in goats, 379

Experimental studies on a Japanese planarian. II. Axial differential in rate of eye formation in reconstitution, 437

Experimental study on the origin of melanophores in birds, The, 48

Experiments on artificial parthenogenesis in starfish eggs, with a review, Further, 239

Gaunt, Robert, 373

Gordon, Albert S., 366

Gregg, E. V., 395

Growth studies of ciliates. V. The acceleration and inhibition of ciliate growth in biologically conditioned medium, 209

Growth studies of *Colpoda duodenaria*. I. Sterilization of the ciliates, 431

Hall, R. P., 384

Hall, Robert H., 193

Hamburger, Viktor, 355

Hartman, F. A., 476

Hastings, E., 384

Heilbrunn, L. V., 281

Helfer, R. G., 296

Inhibition of lactation in the rat, 373

Irritability of frog muscle in relation to the sodium ion, The, 281

Johnson, W. H., 227

Karyoplasmic ratio in the embryos of monovoltine and bivoltine races of the silkworm *Bombyx mori* L., The, 70

Kidder, George W., 209

Lewis, L. A., 476

Lillie, Frank R., 103

Lillie, Ralph S., 239

Lutherman, C. Z., 395

Manifestations of incompatibility in limb grafts made between bird embryos of different species, 136

Maximal density and phases of death in populations of *Glaucoma piriformis*, 384

Melanophore responses in fishes in relation to contrast in the visual field, The, 96

Milford, John J., Jr., 344

Mitochondrial orientations and molecular patterns, 268

Modification of development in the sand dollar by NaCNS and Ca-free sea water, 305

Moore, Carl R., 1

Observation on rodents in Herter's temperature gradient, 186

Oxygen as a factor in the polarity of *Corymorpha palma*, 174

Park, Thomas, 395

Pepper, J. H., 470

Physiology of development of the feather. V. Experimental morphogenesis, 103

POLLISTER, ARTHUR W., 268
PRICE, DOROTHY, 145

Rat prostate and seminal vesicle grafts in relation to the age and sex of the hosts, 145
RIS, HANS, 48
Role of a cut surface in *Tubularia* regeneration, The, 328
Role of the injured surface in the production of the injury potential of the frog muscle, The, 78
Role of sex hormones in sex differentiation in the opossum (*Didelphys virginiana*), On the, 1
ROSE, S. MERYL and FLORENCE C., 328
RULON, OLIN, 305, 461

SCHECHTER, VICTOR, 174
Sequence of roentgen ray effects on the development of the grasshopper egg. I, 163
SHELDON, F. F., 476
SHOTTENFELD, A., 384
STEINBACH, H. BURR, 78
Stimulating action of citrates and oxalates on the *Nereis* egg, The, 84
Studies on homoplastic and heteroplastic transplantation of metanephric primordia to the coelom of the chick embryo, 344
Studies in population physiology. X. Interspecific competition in populations of granary beetles, 395
Study of environmental factors affecting cystment in *Woodruffia metabolica*, A further, 227

TAYLOR, C. V., 431
TIRELLI, MARIO, 70
Transplantation of limb primordia of homozygous and heterozygous chondrodystrophic ("Creeper") chick embryos, 355

WAGTENDONK, W. J. VAN, 431
WALTHER, R. F., 476
WANG, HSI, 103
WATANABE, YISAMU, 316, 437
WIERSMA, C. A. G., 296
WILBUR, KARL M., 84

Volume XIV OCT 21 1941 Number 4
Medical Library

Physiological Zoölogy

OCTOBER, 1941

Studies in Population Physiology. X. Interspecific Competition in Populations of Granary Beetles
Thomas Park, Ella Virginia Gregg, and Catharine Z. Lutherman 395

Growth Studies of *Colpoda duodenaria*. I. Sterilization of the Ciliates
C. V. Taylor and W. J. Van Wagtendonk 431

Experimental Studies on a Japanese Planarian. II. Axial Differential in Rate of Eye Formation in Reconstitution - - - - - - *Yisamu Watanabe* 437

Differential Modifications of Coelom Development in the Starfish, *Patiria miniata*
C. M. Child 449

The Alteration of Developmental Pattern in the Sand Dollar by Pilocarpine
Olin Rulon 461

Buffering Capacity and Composition of the Blood Serum and Regurgitated Digestive Juices of the Mormon Cricket (*Anabrus simplex* Hald.)
J. H. Pepper, F. T. Donaldson, and Ellsworth Hastings 470

Some Blood Constituents of the Normal Skate
F. A. Hartman, L. A. Lewis, K. A. Brownell, F. F. Shelden, and R. F. Walther 476

Index to Volume XIV - - - - - - - - - - - - - - - - 487

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

WARDER C. ALLEE
Managing Editor

EDITORIAL BOARD

J. H. BODINE, *University of Iowa*
C. M. CHILD, *Stanford University*
M. F. GUYER, *University of Wisconsin*
L. V HEILBRUNN, *University of Pennsylvania*
F. L. HISAW, *Harvard University*
E. E. JUST, *Howard University*
F. R. LILLIE, *University of Chicago*
R. S. LILLIE, *University of Chicago*
C. R. MOORE, *University of Chicago*
W. W. SWINGLE, *Princeton University*
C. V. TAYLOR, *Stanford University*
B. H. WILLIER, *Johns Hopkins University*

Physiological Zoölogy is published quarterly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. The subscription price is $7.50 a year; the price of single copies is $2.25. Orders for service of less than a full year will be charged at the single-copy rate. Postage is prepaid by the publishers on all orders from the United States and its possessions, Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Guatemala, Haiti, Republic of Honduras, Mexico, Morocco (Spanish Zone), Nicaragua, Panama, Paraguay, Peru, Rio de Oro, El Salvador, Spain (including Balearic Islands, Canary Islands, and the Spanish Offices in Northern Africa; Andorra), Spanish Guinea, Uruguay, and Venezuela. Postage is charged extra as follows: for Canada and Newfoundland, 20 cents on annual subscriptions (total $7.70), on single copies 5 cents (total $2.30); for all other countries in the Postal Union, 48 cents on annual subscriptions (total $7.98), on single copies 12 cents (total $2.37). Patrons are requested to make all remittances payable to The University of Chicago Press in United States currency or its equivalent by postal or express money orders or bank drafts.

The following are authorized agents:

For the British Empire, except North America, India, and Australasia: The Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1, England. Prices of yearly subscriptions and of single copies may be had on application.

For Japan: The Maruzen Company, Ltd., Tokyo.

For China: The Commercial Press, Ltd., 211 Honan Road, Shanghai.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editors and manuscripts should be addressed to W. C. Allee, Managing Editor of PHYSIOLOGICAL ZOÖLOGY, The University of Chicago, Chicago, Ill.

The articles in this journal are indexed in the *International Index to Periodicals*, New York, N.Y., the *Quarterly Cumulative Index Medicus*, and *Biological Abstracts*.

Applications for permission to quote from this journal should be addressed to The University of Chicago Press, and will be freely granted.

Entered as second-class matter October 27, 1928, at the post-office at Chicago, Illinois, under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in United States Postal Act of October 3, 1917, Section 1103, amended February 28, 1925.

[PRINTED IN U.S.A.]

THE EUGENICS REVIEW

The Biological Quarterly which the Layman can read

Articles, news items and editorial comments on the demographic and eugenic aspects of war and peace, with particular reference to the biological consequences of some of the measures proposed and adopted for the prosecution of the war, will be published in every issue of the *Review*. At the same time every effort is being made to retain the special features—*e.g.* notices of books, abstracts of periodicals, notes and memoranda, and correspondence—which contributed so much to the value and popularity of the *Review* in the pre-war period.

Published quarterly—January, April, July, October

Price: THREE SHILLINGS; or TWELVE SHILLINGS per annum post free, at all high-class booksellers

Free to Fellows and Members

THE EUGENICS SOCIETY,
69 Eccleston Square, S.W.1, London

MACMILLAN & Co., Ltd.,
St. Martin's St., W.C.2, London

"....probably the best in its field."—*Annals of the Entomological Society of America.*

A LABORATORY INTRODUCTION TO ANIMAL ECOLOGY AND TAXONOMY

By ORLANDO PARK, W. C. ALLEE, *and* VICTOR SHELFORD

A revision of the well-known Allee and Shelford *Synoptic Key to the Phyla, Classes, and Orders of Animals*, incorporating a series of exercises with a general approach to natural history from the ecological point of view. Its threefold organization—identification, observation, and experiment—offers a sound and stimulating approach to laboratory work.

233 pages, 28 illustrations, 8vo, cloth, Cercla binding

$2.00

THE UNIVERSITY OF CHICAGO PRESS

PATTERNS AND PROBLEMS OF DEVELOPMENT

By C. M. Child

HERE is the crowning synthesis of the lifetime work of one of the most original thinkers and productive workers in biology. From many years of experimental investigation—and a bibliography of more than 1,600 titles—Child formulates a theory of development on a much broader basis than the "organization" of the egg.

A WORK destined to arouse both enthusiasm and controversy. This summing-up will undoubtedly become a standard work useful to all specialists, and a reference book of broad scope for advanced students of embryology and developmental physiology in general.

811 pages, 224 illustrations, $8.00

THE UNIVERSITY OF CHICAGO PRESS

- **ANATOMY**
- **ANTHROPOLOGY**
- **MORPHOLOGY**
- **NEUROLOGY**
- **NUTRITION**
- **PHYSIOLOGY**
- **ZOOLOGY**

- Journals of The Wistar Institute of Anatomy and Biology merit your careful examination.
- Contributed to and edited by outstanding scientists in the respective fields.

Authoritative! ***Informative!***

Invaluable to the scientist and research worker!

For further information, address

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

Woodland Avenue and Thirty-sixth Street

Philadelphia, Pa.